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(*Continued*)

ROLE OF THE ADRENAL CORTEX IN IMMUNITY*†‡

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IT IS the purpose of this paper to present experimental evidence for the role of the adrenals in immune mechanisms and to attempt to fit existing experimental and clinical data into an over-all working concept of the role of pituitary-adrenal cortical secretion in the allergic state. The term "immunity" is used broadly to include both natural and acquired resistance contributing to the defense of the organism, particularly against intoxications, infectious diseases, and environmental stresses. Immunity may connote increased resistance to both nonantigenic and antigenic substances, although antibody responses to specific antigens will be presented to illustrate in part the relation of the adrenal cortex to immunity.

The term "allergy" is used in its original concept as defined by Von Pirquet,[1] namely, the changed state of the organism in relation to parenterally introduced antigens. This altered state of the organism, due to previous contact with an antigen, is detectable by the inflammatory reaction that follows a second injection of the antigen subcutaneously, as seen in allergic inflammations, or by the systemic general reaction which follows its injection intravenously, as, for example, in anaphylaxis. With these viewpoints, the allergic state is one segment of the broad field of immunity. Let us consider first the broader aspects of immune mechanisms, and then turn our attention to the allergic state.

The factors which contribute to immunity, both natural and acquired, have been recognized and partially evaluated as a result of experimental and clinical studies of the past sixty years. Among the more important of these factors are the following: (1) genetic, (2) cellular, (3) nutritional, (4) environmental, and (5) hormonal.

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The present discussion will be concerned chiefly with the relation of one endocrine gland, the adrenal cortex, to immunity. However, as will be evident, the level of functioning of the adrenal cortex is influenced markedly by at least two other of the above-mentioned factors, namely, nutrition and stimuli present in the environment. In addition, the adrenal cortex bears an intimate relationship to yet another factor which contributes to immunity, namely, the cellular factor. Thus, the activity of the adrenal cortex, although only a single endocrine influence, is integrated with several other of the factors of importance in immunity.

The relation of the adrenals to the defensive mechanisms of the body, particularly against intoxications, infectious diseases, and environmental stresses of the type of anoxia and abnormal temperatures, has been established generally from both morphological and physiological studies. Moreover, the pathologists have long suspected the active participation of the adrenal cortex in these circumstances. Hyperemia, edema, hemorrhage, and focal necrosis occur in the adrenal glands following burns, food or chemical poisoning, and acute infections. Conversely, adrenalectomized animals and patients with Addison's disease are exceedingly sensitive to drugs, toxins, and stresses in amounts and degree generally innocuous to the normal individual. The established sensitivity of adrenalectomized animals to anesthetics and surgical trauma is now being appreciated in connection with operative procedures in human subjects.[2]

A precise description of the role of the adrenal cortex in immune mechanisms has been delayed and partially obscured by the numerous immunological studies which have been conducted with the hormone of the adrenal medulla, namely, epinephrine. The basis of this confusion is now apparent as a result of the observation[3] that epinephrine is a powerful augmentor of the rate of secretion of the hormones of the adrenal cortex. The normal mechanism concerned with adrenal cortical secretion is based upon the elaboration of the adrenocorticotrophic hormone, or ACTH, of the anterior pituitary gland. This pituitary hormone is secreted in greater than normal quantity following exposure of the organism to a wide variety of unrelated stimuli: inanition; alterations in environmental factors, for example, cold, heat, and anoxia; trauma in the form of tissue injury, fractures, and hemorrhage; injections of bacterial toxins, toxic chemicals of the type of arsenic or benzene; anesthetics, such as Nembutal; foreign proteins; and a variety of substances normally produced in the organism, for example, histamine, epinephrine, thyroxine, and estrogens.[4] The increased release of adrenal cortical steroid hormones, as a consequence of any of these stimuli, does not occur in the absence of the pituitary, and each of the stimuli, therefore, has a common physiological basis, i.e., its capacity to increase the output of pituitary adrenocorticotrophic hormone. Thus, an increased secretion of epinephrine in the normal individual will elevate the rate of release of pituitary ACTH and result in an increased rate of secretion of the hormones of the adrenal cortex. There is, therefore, an intimate physiological relationship between the secretions of the two endocrine glands which constitute the adrenal, namely, the adrenal medulla and the adrenal cortex. It is quite likely that data establishing either an increased immunity or an alteration in the allergic state fol-

lowing epinephrine injection were a result in part of the increased concentration of circulating adrenal cortical steroid hormones resulting from the ability of epinephrine to augment pituitary ACTH secretion. Of considerable interest in the field of allergy is the fact that a single injection of an antigen has also been demonstrated[5] to cause an augmented release of pituitary adrenocorticotrophic hormone. The immunization process, therefore, includes among other things the raising of the circulating level of adrenal cortical steroids.

One additional group of recent experimental facts is of some interest and significance in relation to the secretion of pituitary adrenocorticotrophic hormone. The recent observations of several investigators, notably Harris[6] working in England, have shown that the rate of release of ACTH by the anterior pituitary is influenced markedly by the stimulation of specific hypothalamic centers. Harris has postulated a neural humoral mechanism by which stimulating influences from the hypothalamus may be transmitted to the anterior pituitary gland, with a resultant release of ACTH. The data of Harris, together with those of Markee and his associates[7] at Duke University, point to the adrenergic nature of the neural humoral hypothalamic factor. There begins to evolve, therefore, some understanding of the mechanism of the action of nervous system activity and factors altering its level of activity, e.g., emotional or psychic factors, on the secretion of pituitary adrenocorticotrophic hormone.

From the foregoing, it would appear that any physiological process, which contributes to an immune mechanism and is influenced or altered specifically by adrenal cortical secretion, will also be affected by any one of a wide variety of stimuli which augment pituitary-adrenal cortical activity. This general body of information will be of considerable aid in explaining the similarity of certain phenomena of immunity under widely diverse circumstances.

Let us turn now to the question of what pituitary-adrenal cortical stimulation, and the consequent increase in circulating adrenal cortical steroid hormones, might contribute to immune mechanisms. The answer to this question can be derived in part from the established physiological functions of these steroid hormones, which affect a wide variety of important metabolic phenomena. It will be recalled that the hormones of the adrenal cortex alter water and salt metabolism, protein metabolism, and carbohydrate metabolism. In addition, it has been demonstrated recently that adrenal cortical steroids contribute to certain aspects of immune cellular responses, including the development of phagocytic cells, the production of immune globulin, and an increased resistance of tissues to the penetration of foreign substances. Although it is not established clearly that the known physiological effects of pituitary-adrenal cortical secretion can explain completely the role of this endocrine mechanism in immune reactions, the available evidence does afford a basis for both the present discussion and future investigation. The recent observations of the clinical efficacy of ACTH in a wide variety of disordered states have greatly extended the use of this hormone. Particular aspects of ACTH therapy will be discussed by subsequent speakers. However, it should perhaps be emphasized at this time, and reiterated at frequent intervals, that pituitary ACTH has a number of very diverse and profound effects on many body cells. These effects have attended

the use of this hormone in a variety of clinical studies, in which undesirable accentuation of normal physiological reactions have occurred as a result of the chronic administration of the hormone. Certain of these effects may be referred to by subsequent speakers.

Of the cells concerned with immune mechanisms, the role of reticulo-endothelial cells has been known since the fundamental observations of Metchnikoff,[8] initiated in the latter part of the last century. Metchnikoff's classical studies demonstrated clearly the significance of the macrophage and of phagocytosis in immune mechanisms contributing to general resistance. This was true not only in relation to the invasion of the body by infectious organisms, but also with respect to the defense against soluble toxins or toxic chemical agents. The function of the macrophage in immune mechanisms appeared to be dependent upon the phagocytic capacity of the cell, which in turn is apparently of importance in the possible role of these cells in the formation of antibodies. However, even in the case of toxic nonantigenic material, for example, potassium arsenite, Metchnikoff showed clearly that the degree of resistance exhibited by the organism against arsenic poisoning was directly proportional to the extent of macrophage development.

Another cell type with an established role in immune mechanisms is the lymphocyte. This cell, like the macrophage, arises from the reticular structures and participates in chronic inflammatory processes of all types, and accumulates at the site of chronic irritation.

In view of these established roles of the macrophage and the lymphocyte in immune mechanisms, it is of considerable interest and significance that the adrenal cortical steroids have a marked effect on these two types of cells and on reticulo-endothelial cells in general. Increased numbers of phagocytic cells develop in lymphoid structures as a result of increased adrenal cortical steroid secretion or injection.[9] Indeed, the histological picture seen in these circumstances is similar in many respects to that observed following a single injection of a bacterial antigen.[10] This ability of adrenal cortical steroids to increase the number of macrophages in tissues is a striking contribution of pituitary-adrenal cortical secretion to immune mechanisms. The physiological importance of this had been illustrated by the observations of Reiss and Gothe[11] and, more recently, of Gordon and Katch.[12] Both of these groups of investigators have shown that the capacity of the reticulo-endothelial system to remove soluble or particulate material from the circulation can be augmented as a result of increased pituitary-adrenal cortical activity.

The structure and, therefore, presumably, the function of the lymphocyte, is also affected markedly by adrenal cortical steroids. Lymphocyte dissolution and degeneration, with a resultant decrease or involution of lymphoid tissue, is a well-known phenomenon occurring following a wide variety of stresses. These alterations in lymphocyte structure and numbers can be produced[9] either with pituitary ACTH or by the administration of adrenal cortical steroids, oxygenated in position 11 of the steroid nucleus. It is of some interest, particularly in view of current interest in the therapeutic use of adrenal cortical steroids, that desoxycorticosterone acetate, in the doses employed, does not affect lymphoid tissue

structure or function. It seems clearly indicated, moreover, that in most instances the wide variety of stresses which cause augmented tissue involution have their physiological basis in the effect of this stress in augmenting the rate of pituitary-adrenal cortical secretion. This is strikingly illustrated, for example, by the involution of lymphoid tissue which occurs in fasted animals but not in fasted animals which have been previously adrenalectomized.[13] Indeed, it may be pointed out that there are at present only a very few agents which are known to cause alterations in lymphoid tissue size and function in the absence of the adrenals. These agents are: x-ray irradiation, the nitrogen mustards, urethane, and large doses of estrogens.[14] It is reasonable to conclude, until evidence to the contrary is presented, that the wide variety of other agents which affect lymphoid tissue do so as a result of their influence on pituitary-adrenal cortical secretion. This may explain the relative state of flux which is seen in lymphoid tissue and the infrequency with which lymph nodes of normal histologic appearance are encountered.

With regard to the contribution of the lymphocyte to immune mechanisms, it has been demonstrated that in the immunized animal one of the constituents of lymphocytes is antibody globulin.[15-17] In the normal as well as the immunized animal, the shedding of lymphocyte cytoplasm and lymphocyte dissolution may be accelerated as a consequence of increased pituitary-adrenal cortical secretion, and the constituents of this cell thus released to the lymph and eventually participate in metabolic transformations.[18, 19] Therefore, the normal physiological functioning of the lymphocyte may be accelerated by the administration of adrenal cortical steroids or as a result of the release in the organism of excessive amounts of pituitary ACTH following any one of the wide variety of stimuli which have been mentioned previously. This is seen, for example, in the fact that the injection of adrenal cortical steroids, or increased secretion of these hormones as a result of pituitary adrenotrophic hormone injection, causes an elevation of antibody titer, either in a hyperimmunized animal or in a previously immunized animal with little circulating antibody. These experiments[18, 19] suggest that the physiological basis of the anamnestic response is the pituitary-adrenal cortical control of lymphocyte structure and function. This would afford an explanation for the anamnestic response which is produced by a wide variety of unrelated stimuli.[20]

These results with antibody production in the intact animal have been extended recently to an examination of the role of the adrenal cortex in the production of tissue antibodies in vitro. This approach was suggested for two reasons. First, in studying isolated tissues, the variables affecting the concentration of antibody in the circulation at a particular time would be largely eliminated. Second, the relation of the inflammatory reaction to local tissue antibody production warranted detailed study of the process.

The experimental procedure developed for studying antibody production by isolated tissues in vitro is as follows: Tissues taken from previously immunized animals are minced, and immediately incubated under physiological conditions in nonimmune homologous serum. The antibody released by the tissue to the serum can be titrated, as can the antibody in the tissue prior to, and at the end

of, the incubation period. This recent work,[21] which has been done in collaboration with Roberts and Adams, suggests that the actual site of antibody production may vary with the mode of immunization employed. When rats or mice are immunized with a single intravenous dose of sheep erythrocytes, the spleen appears to be the most active source of antibody. On the other hand, when the antigen is given in periodic intraperitoneal injections and various tissues taken from the rats at the height of antibody production, the most active release of antibody in vitro is observed in incubates of mesenteric lymph nodes. This technique has now been extended to study[5] the effect of the adrenal cortex on tissue antibody production. When a comparison is made of the in vitro production of hemolysins against sheep erythrocytes by splenic mince obtained from adrenalectomized and control rats injected intravenously 5 days earlier with a single dose of sheep red blood cells, there is no indication of new formation of antibody in splenic tissue from immunized adrenalectomized rats. However, under the same conditions, tissues of unoperated immunized animals show a continuing production of antibody during the incubation. In further experiments, it has been demonstrated that tissues from immunized animals, which had been injected previously with adrenal cortical hormone, show a degree of antibody production which exceeds that of nonhormone-treated, immunized animals.

The question arises whether antigen-antibody reactions in tissues are limited to those tissues in which actual synthesis of antibody occurs. This would not seem likely in view of extensive experience in the field of passive sensitization. This suggests a rather general capacity of tissues for antibody uptake and is confirmed experimentally[21] by the simple procedure of incubating tissues from nonimmunized rats with antibody-containing serum from immunized rats. In all instances the antibody content of the serum medium decreased, and antibody was apparently taken up by all tissues studied. The influence of the adrenal cortex on this process remains to be investigated and will be of interest in relation to a possible role of the adrenal in passive sensitization and immunity.

It is clear, therefore, that the adrenal cortex plays the following roles in influencing immune mechanisms: (1) The level of secretory activity of the adrenal cortex is influenced by the administration of an antigen, since antigens may augment, in a nonspecific manner, pituitary-adrenal cortical secretion. (2) An increased level of circulating adrenal cortical steroids has a profound effect on cells of the reticulo-endothelial system, namely, macrophages and lymphocytes, which are possibly concerned with the processes of antibody formation and release. (3) The rate of formation of antibodies by specific tissues is markedly influenced by adrenal cortical secretion.

Recent observations have revealed yet another role of the adrenals in immune mechanisms. This role is concerned specificially with the phenomenon of tissue permeability. In collaboration with Opsahl and Duran-Reynals, it has been demonstrated[22, 23] that the intradermal spreading of India ink, mixed with either saline or testicular hyaluronidase (spreading factor), is inhibited by adrenal cortical extract. This inhibition is produced when the extract is administered either intraperitoneally or intradermally at the site of India ink injection. When administered locally, the greatest effect of adrenal cortical

extract is obtained when it is given simultaneously with the India ink. The inhibitory effect of the hormone decreases progressively with lengthening intervals between injections of adrenal cortical extract and India ink. On the other hand, adrenalectomy resulted in a marked enhancement of the spreading reaction when this took place in the living animals. On the basis of these observations, it seems clear that adrenal cortical function may play a profound role in resistance against invasions of the organism by toxic substances or organisms. It would appear that the adrenals are concerned in the maintenance of the tonus of the ground substance of the mesenchyme. This may be of significance in relation to certain of the clinical syndromes which appear to be ameliorated following ACTH or cortisone therapy.

In concluding our discussion of the role of the adrenals in immune mechanisms, reference should be made to the hypersensitive state. This appears to be indicated, particularly in view of the remarkable reports which are becoming available of the therapeutic effects of adrenocorticotrophic hormone and potent adrenal cortical steroids in diseases in which a hypersensitivity may be an etiological factor. The direct relationship of the adrenal cortex to one manifestation of the hypersensitive state, namely, anaphylaxis, has been established clearly. Normal mice and rats, which are exceedingly resistant to the induction of the anaphylaxis phenomenon, become highly susceptible following adrenalectomy.[1] The basis of the role of the adrenal cortex in the prevention of anaphylaxis is not yet clear. Understanding of this mechanism will undoubtedly contribute to an understanding of the role of the adrenal cortex in the hypersensitive state in general.

A few comments should also be made in relation to the effect of pituitary-adrenal cortical secretion in causing disappearance of blood eosinophils.[24] In view of the eosinophilia in certain allergic states and the eosinophilic infiltration into subcutaneous connective tissue, which occurs as a result of inflammation, it is of considerable interest that augmented pituitary-adrenal cortical secretion results in a marked diminution in the level of circulating blood eosinophils. The basis of this eosinopenia is still unexplained; it is unknown whether it is due to a failure of production of these cells, an increased rate of their removal by the tissues, or to an increased destruction of eosinophils. If it should be observed that there is an increased infiltration of eosinophils into the connective tissues as a result of adrenal cortical extract administration, as occurs during the acute inflammatory response, there would be established a connecting link between the adrenals and the allergic type of inflammatory reaction. However, there yet remains unexplained the nature of the mechanisms by which adrenal cortical steroids ameliorate the hypersensitive state. Perhaps several speculations may be permitted at the present time, with the purpose chiefly of indicating possible further lines of investigation in this fundamentally important field. It would appear logical to suggest that pituitary-adrenal cortical secretion may affect the antigen-antibody reaction, which is a preliminary to allergic inflammation; this action of the hormonal mechanism may be based on one or more of at least three processes: (1) an alteration in the relative concentrations of antigen and antibody in the tissues,

thus affecting their rate of interaction; (2) an alteration in tissue factors influencing the extent of combination of antigen and antibody, and (3) an alteration in the degree of production of tissue inflammatory substances released as a result of antigen-antibody reaction. It would at the moment seem paradoxical that the inflammatory response resulting from antigen-antibody reaction may be blocked by adrenal cortical steroids, which also augment production of tissue antibody. This may indicate that the adrenal cortical steroids are concerned with the inflammatory response in some manner other than that relating to the reaction of antigen with antibody. This consideration appears to favor at present the working hypothesis that the adrenal cortical steroids block the tissue production of substances which are deleterious to cells and cause cellular injury, and thus function in ameliorating the hypersensitive state.

In concluding these remarks, it may be well to direct attention again to the very profound and widespread physiological effects of the secretions of the adrenal cortex. It is perhaps desirable to re-emphasize these in view of the high degree of enthusiasm and possible eventual general use of either ACTH or adrenal cortical steroids. On the basis of the known physiological effects of these hormones, it is theoretically possible to produce the following undesirable effects: (1) pancreatic diabetes, (2) osteoporosis, (3) hypertension, (4) Cushing's syndrome-like symptoms, and (5) marked accentuation of physiological effects characteristic of male hormone production, e.g., masculinization in the female. Finally, it should be emphasized that these hormones have a profound catabolic effect on nitrogen metabolism, and their chronic use should be accompanied by an adequate caloric intake and perhaps avoided in states of extreme malnutrition.

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THE EFFECT OF CORTISONE ON BRONCHIAL ASTHMA AND HAY FEVER OCCURRING IN SUBJECTS SENSITIVE TO RAGWEED POLLEN* †

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A STUDY has been undertaken to determine whether or not cortisone will influence bronchial asthma and hay fever resulting from sensitivity to ragweed pollen. A number of general facts have suggested the possible value of such a study. Experimental data have interlinked three systems and mechanisms of the body: the adrenal cortex, lymphoid tissue, and reactions of immunity.

A pituitary-adrenal control influences the occurrence and severity of anaphylactic shock by enhancing resistance to this form of trauma.[1, 2] The tolerance to the deleterious effects of histamine is decreased when an animal is deprived of the function of its adrenal cortex.[3-6]

The transport and, likewise, the possible formation of antibodies in lymphoid tissue[7-10] is significant when considered with the fact that the adrenal hormones can effect a degeneration of lymphoid tissue.[11, 12]

Evidence suggests that the adrenal cortex can influence the titer of circulating immune bodies, and that extracts of this gland can bring about an anamnestic response.[13]

In this study of the allergic state we have selected for consideration the adrenal cortical fraction cortisone (17-hydroxy-11-dehydrocorticosterone). Among the several hormones of the adrenal cortex cortisone has been demonstrated to be effective in bringing about lymphocytic degeneration in tissue culture experiments,[12] in neoplastic lymphoid tumors,[14, 15] and possibly in circulating lymphoid tissue.[16] Cortisone, and adrenal cortical extracts containing cortisone, will effect an anamnestic response.[13] Cortisone is in the 11-oxysteroid fraction of adrenal cortical extract which generally has been effective in bringing about lymphocytic degeneration and in evoking an anamnestic response.[13]

As diseases for consideration in this study of the allergic state we have selected seasonally occurring bronchial asthma and hay fever. Among allergic disorders these diseases are unique in affording suitable methods of control in such a study. Symptoms may be severe, are of relatively constant occurrence, and afford a means of objective evaluation. The symptoms experienced in pollinosis are of sufficient duration to afford an opportunity for both control and test studies to be made on a single subject. The degree of exposure of a patient to an offending allergen may be estimated with reasonable accuracy by a day-to-day study of the pollen content of the air. Among the allergic dis-

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orders pollinosis is a most nearly "pure" extrinsic form of allergy. Complicating infections and neurogenic factors are less important than may be the case in perennial vasomotor rhinitis, perennial forms of asthma, and the allergic dermatoses.

The use of cortisone in the treatment of hay fever or less severe bronchial asthma would not be justified under present circumstances of supply and understanding. However, the advantages, as indicated, seem to justify its use in these diseases as a means of studying the mechanism of the allergic state.

As volunteer patients for this study, 3 persons having both bronchial asthma and hay fever resulting from sensitivity to ragweed pollen were selected. Such patients were given an injection either of cortisone* or of cholesterol into the gluteal muscles. A suspension of 100 mg. was administered daily over a 4-week period starting Aug. 19, 1949. The preparations which we administered were in containers labeled only by a code number. As the cortisone and cholesterol suspensions were grossly identical in appearance, the knowledge as to which preparation was injected remained unknown to the patients and to the physicians during this study. Not until the conclusion of the study, when all observations had been made and recorded, was information concerning the dates of test and of control periods available to us. In this manner objectivity was assured. While changes from test to control suspensions were made periodically in the course of these studies, at no time was a substance given for a period of less than 6 consecutive days. By this procedure errors in interpretation as a result of delayed absorption of a suspension were minimized.

The patients in this study were observed daily by physicians from both medical and otorhinolaryngologic sections. In evaluating and classifying symptoms, several criteria were employed. The occurrence and severity of asthma in the preceding 24-hour period were evaluated, as were the symptoms of paroxysmal sneezing, nasal congestion, rhinorrhea, and conjunctival irritation. With respect to hay fever, a classification of symptoms on the basis of 1 to 4 was employed wherein grade 1 has designated minimal difficulty and grade 4 has designated maximal difficulty. Each patient selected for study was engaged in an occupation assuring a generous exposure each day to pollen-laden air.

The appearance of the intranasal and conjunctival mucous membranes was studied particularly for changes in the degree of edema, pallor, and moisture.

In addition to studies directed toward an evaluation of asthma and hay fever, the patients were observed for evidence of any untoward reaction to cortisone. Frequent determinations of blood pressure and clinical observations for disturbances of electrolyte metabolism were made. Weekly determinations of the leukocyte count and differential leukocyte count were made, particular attention being directed to the relative percentage of lymphocytes and eosinophilic leukocytes.

CASE REPORTS

CASE 1.—A 60-year-old man, whose occupation was that of an automobile dealer, for 35 years had had hay fever which for the past 12 years had been associated with the occurrence of bronchial asthma. His symptoms of hay fever and asthma had occurred only in the weeks between mid-August and the period immediately following a frost. An allergy

*The acetate derivative of cortisone was employed throughout the study.

survey including common inhalant allergens and pollens prevalent in the season of his difficulty revealed sensitivity to ragweed pollen. From 17 to 10 years prior to this study, the patient had received yearly hyposensitization with an extract of mixed ragweed pollen with an estimated 50 to 75 per cent relief from symptoms of hay fever. In recent years the patient had employed numerous remedies including large doses of ascorbic acid and numerous antihistamine drugs with partial relief of symptoms. With the exception of asthma and hay fever the patient was in excellent health.

At the time when cortisone was first administered, this patient was having severe symptoms resulting from hay fever and was having daily bronchial asthma. After the administration of cortisone the symptoms of hay fever subsided to a minimal level within 3 days and those of asthma disappeared within 1 day. During this time the concentration of pollen in the air remained high. The patient had relief from symptoms for 4 days extending into the control period. The relief of symptoms early in the control period may be ascribed to the delayed absorption of suspended cortisone from the site of its injection. On the fourth day of the control period both hay fever and asthma recurred. With the resumption of administration of cortisone, either minimal or no symptoms of hay fever were experienced and no asthma occurred. No other drugs for symptomatic relief were employed during this study (Fig. 1).

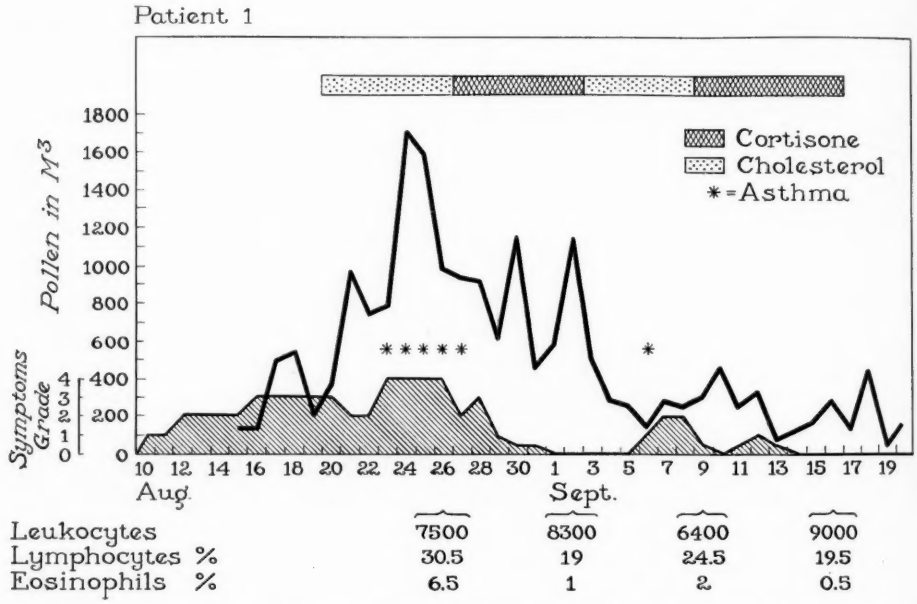
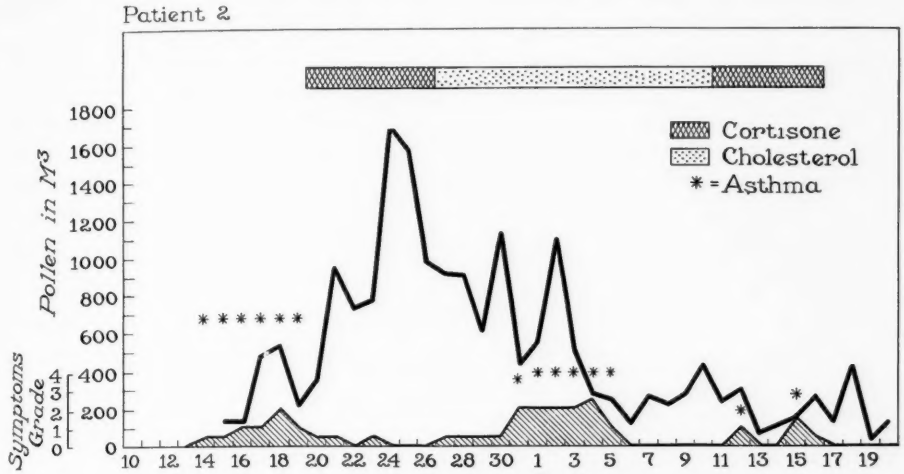


Fig. 1.—The effect of cortisone on bronchial asthma and hay fever resulting from sensitivity to ragweed pollen in Patient 1.

CASE 2.—A 57-year-old man, who was engaged in office work in an executive capacity, had had seasonal hay fever occurring between mid-August and frost since childhood. In the 10 years preceding this study an associated asthma had occurred in this season. Both hay fever and asthma were virtually entirely confined to this season. An allergy survey revealed sensitivity to ragweed pollen. In the years 1930 to 1932 he had received hyposensitization with ragweed pollen extract with an estimated 50 per cent relief of symptoms. In recent years considerable symptomatic relief had been obtained with various antihistamine drugs. The patient was otherwise in excellent health.

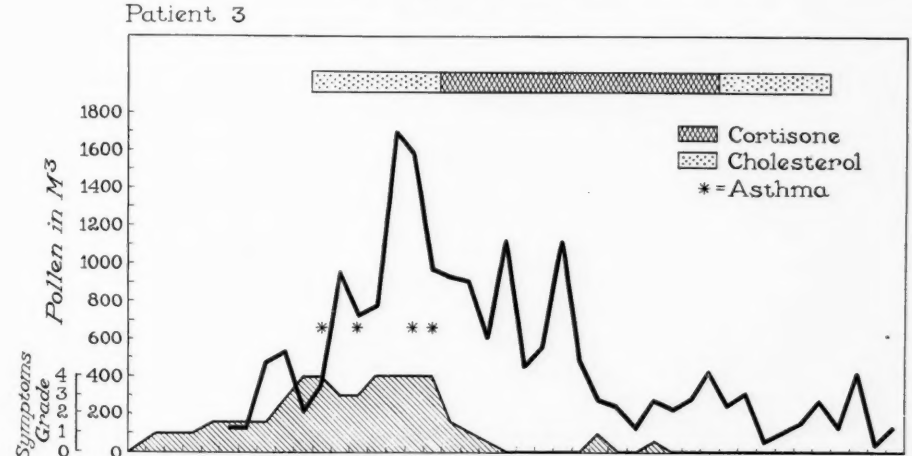
At the time when the second patient first received cortisone, moderate symptoms of hay fever were present as well as daily asthma for 6 consecutive days. With the administration of cortisone no further asthma and only minimal hay fever occurred until the fourth day of

the control period. On that day hay fever of moderate severity developed as well as daily bronchial asthma until the patient's symptoms abated at a time when the pollen content of the air dropped precipitously. With the resumption of administration of cortisone minimal



Leukocytes	6600	7000	4600	8700
Lymphocytes %	25	30	43	31.5
Eosinophils %	1	2.5	2	0.5

Fig. 2.—The effect of cortisone on bronchial asthma and hay fever resulting from sensitivity to ragweed pollen in Patient 2.



Leukocytes	7300	8900	9200	8400
Lymphocytes %	30	15	17.5	22.5
Eosinophils %	5.5	2	1	4.5

Fig. 3.—The effect of cortisone on bronchial asthma and hay fever resulting from sensitivity to ragweed pollen in Patient 3.

hay fever and minimal wheezing occurred for 1 day on two occasions. Exertion in unseasonably cold weather with a bronchitis not yet controlled may explain this occurrence. No other drugs for symptomatic relief were employed during this study (Fig. 2).

CASE 3.—A 57-year-old woman, a nurse, had had seasonal hay fever for approximately 20 years. This had been associated with bronchial asthma virtually since its onset. The hay fever and asthma began in mid-August and were improved following a frost. An allergy survey revealed sensitivity to ragweed pollen. In recent years asthma had persisted until November. Hyposensitization with mixed ragweed pollen extracts was of little benefit when used in 1935. The patient had wished no further attempts at hyposensitization. In recent years considerable symptomatic relief had been obtained from antihistamine drugs.

This patient was having extremely severe hay fever associated with bronchial asthma at the time when cortisone was first administered. After she began to receive cortisone no further asthma was experienced. The symptoms of hay fever were completely controlled and recurred in a minimal and transient form on but 2 occasions during the remainder of the period of study. Before symptoms were controlled following administration of cortisone the patient used antihistamine therapy to enable her to continue her work as a nurse (Fig. 3).

COMMENT

Each patient in this study experienced great relief during and immediately following the administration of cortisone from those symptoms associated with bronchial asthma and hay fever. This relief was greater than that previously achieved with hyposensitization to ragweed pollen extract or with the use of antihistamine drugs. The relief of symptoms was largely confined to the period when cortisone was being administered. The delayed absorption of injected suspended medication in all probability explains the period of relief continuing for approximately 2 or 3 days following the last administration of cortisone.

Changes in the appearance of the nasal mucosa were gradual in their occurrence. The relief of the symptoms of hay fever following the administration of cortisone was associated with a decrease in the amount of the secretion bathing the nasal mucosa, a moderate change in the amount of swelling of the middle turbinates, and a slight decrease in the pallor of the mucosa. Further reduction in the edema and pallor continued slowly over several days. The only day-to-day objective change in the nasal mucosa considered to be significant was in the moisture of this membrane. This was well correlated with symptomatic changes.

While none of these patients exhibited eosinophilia in excess of 6.5 per cent, there was in each instance a lowering of the relative eosinophil count at times when cortisone was being administered. No untoward symptoms occurred as a result of administration of cortisone. On occasions those receiving the drug appeared to have slight psychic stimulation. This was, however, indefinite in its occurrence and transitory in its duration.

In many ways ACTH and cortisone bring about parallel changes within the body. The injection of ACTH leads to the release by the adrenal cortex of cortisone-like steroid hormones. Other closely related substances, notably compound F (17-hydroxy-corticosterone), are likewise liberated. The ratio of effective doses of ACTH to cortisone is probably not constant but varies with the amount of available cortisone-like steroid hormones present in the body at a given moment. One hundred milligrams of cortisone is in a general way the equivalent of approximately 40 mg. of ACTH.

SUMMARY

1. Three patients received injections of cortisone during the season when ragweed pollen, to which they were allergic, was in the air.

2. Each patient experienced prompt relief from the symptoms of bronchial asthma and hay fever occurring as the result of that pollen sensitivity.

3. The symptoms of bronchial asthma were relieved more quickly than were symptoms of hay fever.

4. Within 3 days following the onset of administration of cortisone, only occasional allergic symptoms, which were of a mild and transitory nature, were observed.

5. The relief of symptoms was apparently confined to the period during and immediately following the time when cortisone was being administered.

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THE EFFECT OF CORTISONE ON BRONCHIAL ASTHMA*

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THE beneficial effects of ACTH in rheumatoid arthritis,[1, 2, 3] asthma, hay fever, and other allergic disturbances[4-9] prompted a study of the relative effectiveness of cortisone in allergy.

Five patients with severe incapacitating bronchial asthma from 7 to 18 years' duration were selected for therapy with cortisone acetate (Merck). All patients were hospitalized for at least 48 hours prior to starting treatment during which time aminophyllin was given as necessary for relief of severe symptoms, epinephrine being withheld if possible. Dietary or other environmental changes were not made during or immediately following this treatment.

Serial vital capacity determinations, the expiratory rate as modified after the technique described by Hamburger[10] which measures the time required to exhale an arbitrarily selected volume of air, the amount being chosen in relation to the individual's vital capacity and expressed in terms of the cubic centimeters of air expired per second, and direct counting chamber eosinophil and differential counts using the propylene glycol stain technique[11] were made prior to, during the course of, and following cortisone therapy.

In some instances placebo intramuscular injections of saline and propylene glycol were given prior to starting treatment with cortisone; in the others, negative clinical responses had followed a similar treatment program employing concentrated adrenal cortex extract (Upjohn)[12] or some other similar agent either prior to or following treatment with cortisone acetate. All cases were treated with 300 mg. of cortisone intramuscularly. An initial dose of 200 mg. was followed at 24 and 36 hours by additional 50 mg. doses in each of 3 patients. The other two received 50 mg. every 6 hours.

CASE REPORTS

CASE 1.—R. H., 40-year-old man, had been subject to perennial bronchial asthma for the past 17 years and atopic dermatitis since infancy. Incapacitating asthma had been present for 4 months prior to starting cortisone therapy, the last 2 months of which he had been hospitalized and treated by means of symptomatic measures. Although generally his eczema improved coincident with accentuations of his asthma, he continued to have moderately severe pruritic dermatitis of his arms and chest for several weeks before starting cortisone. Aside from house dust treatment, which had not changed the course of his chronic symptoms, no other specific allergic diagnostic procedures or other types of therapy were given.

As shown in Fig. 1, house dust treatment was continued throughout the period of observation. Forty-eight hours after the initial injection of 200 mg. of cortisone he noticed some lessening of his asthma and was able to stop the continuous use of oxygen. A striking objective improvement began during the third day after starting treatment, as may be noted by the sharp increases in his vital capacity and expiratory rate determinations. He continued to show a gradual but progressive improvement in both the asthma and dermatitis throughout the remainder of his hospitalization and remained without troublesome asthma or dermatitis for a total period of 3 weeks, requiring no further intravenous aminophyllin after the first

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week. For an additional month he remained ambulatory and during this interval his intermittent attacks of mild asthma were promptly relieved by the use of epinephrine 1:100 by inhalation.

This patient was subjected to measures for the specific diagnosis of his allergic reactions upon the recurrence of asthma of the former degree of severity. He was found clinically sensitive to corn, wheat, onion, lettuce, and celery as a result of performing individual food tests[13] with the major articles of the diet. He subsequently experienced sustained relief of his asthma and dermatitis by the avoidance of these foods and the use of tolerated foods on a rotating schedule.[13]

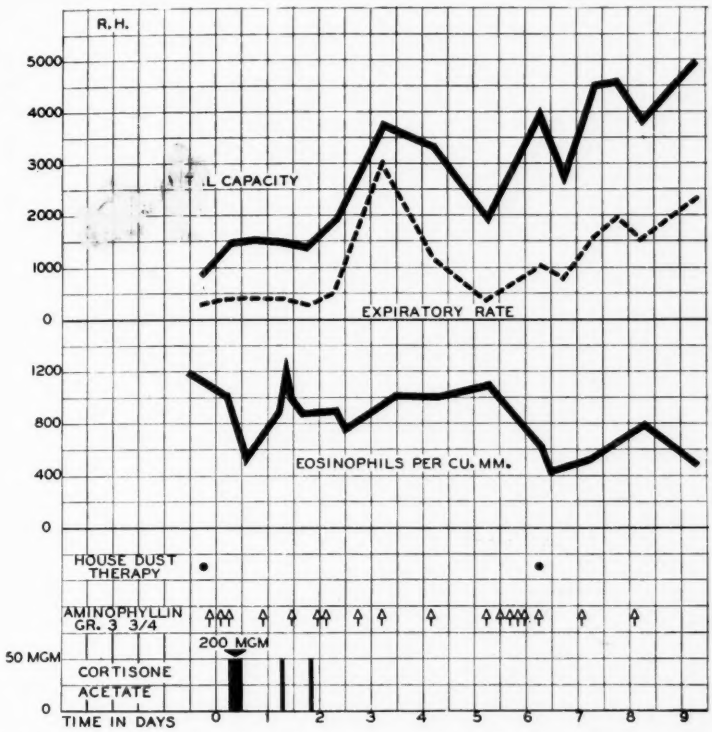

Fig. 1.—Variations in the vital capacity, expiratory rate, and peripheral blood eosinophils in R. H., a patient with bronchial asthma and atopic dermatitis, following cortisone therapy.

Case 2.—B. D., an undiagnosed and previously untreated 45-year-old woman, had been subject to seasonal ragweed hay fever and perennial bronchial asthma for the past decade. The latter had been present to an incapacitating degree for 5 years, confining her to bed much of the time. There was x-ray evidence of moderate pulmonary emphysema, suggestive changes of pulmonary fibrosis, and a residue of pleurisy at the right lateral base. There was also an extensive scar resulting from surgical drainage of a previous empyema. She was known to be exquisitely sensitive to aspirin and sulfonamides.

This patient was hospitalized for a period of 5 days prior to starting therapy with cortisone acetate (Merck). Epinephrine, previously taken regularly, was discontinued 48 hours prior to the administration of cortisone, during which time aminophyllin was given intravenously as necessary and placebo intramuscular injections were started. As may be noted from Fig. 2, the latter had no effect on either the symptomatology or blood findings. Coincident with the administration of 200 mg. of cortisone acetate, she developed a decrease in the circulating eosinophils and subjective evidence of improvement, although

the latter did not influence appreciably the ventilatory determinations. Three days after starting cortisone therapy she was able to stop the continuous use of oxygen but required intermittent treatment with aminophyllin and epinephrine.

Because of the relatively unfavorable response to the course of 300 mg. of cortisone administered in a 48-hour period, this patient was given ACTH, 7 days after starting cortisone, receiving a total dosage of 225 mg. in divided doses over a period of 48 hours. She was observed in the hospital for 1 week after stopping ACTH. During this period she remained relatively free of asthma, receiving symptomatic medications only occasionally, and her vital capacity averaged between 2,500 and 2,700 c.c. with an expiratory rate between 800 and 1,000 c.c. per second. This degree of improvement continued for another week after leaving the hospital. She then had a gradual recurrence of asthma reaching its former degree of severity.

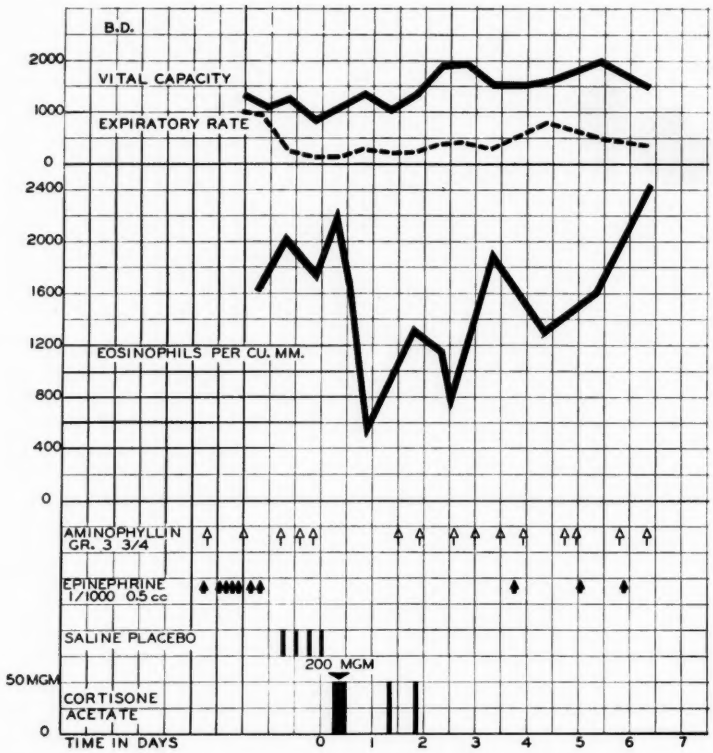

Fig. 2.—Variations in the vital capacity, expiratory rate, and peripheral blood eosinophils in B. D. following cortisone therapy.

CASE 3.—M. H., a 56-year-old nurse, had been subject to perennial bronchial asthma and seasonal and perennial allergic rhinitis complicated by nasal polypi for a decade. A progressive rheumatoid arthritis of her hands began 5 years ago. She remained refractory to conventional allergic management and the severity of her asthma may be judged by the fact that on 2 occasions she sustained fractured ribs during acute asthmatic paroxysms. For many months she had taken over 30 gr. of aminophyllin and between 8 and 10 subcutaneous injections of epinephrine daily in addition to the liberal use of Isuprel by inhalation. Roentgenograms of the chest showed advanced pulmonary emphysema and healed fracture deformities of her ribs.

Epinephrine was withheld 24 hours prior to the initial injection of 200 mg. of cortisone acetate. However aminophyllin was continued for relief of symptoms as indicated in

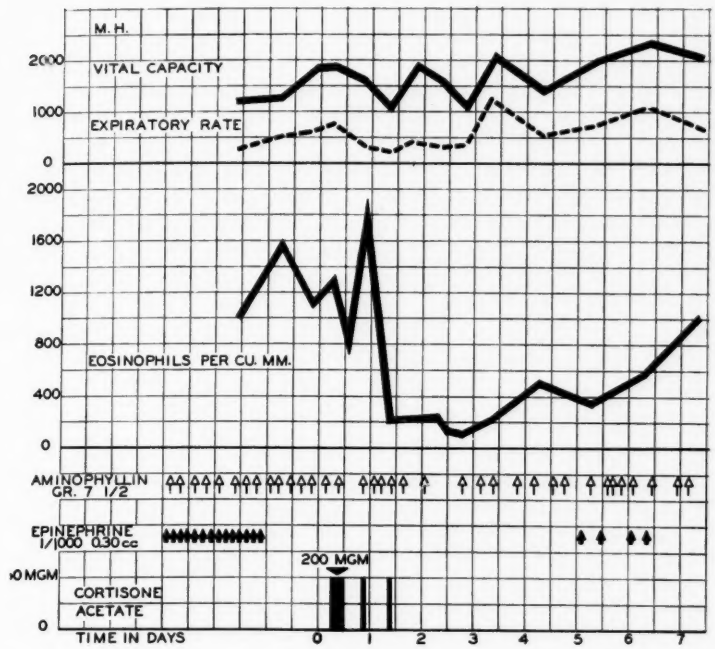

Fig. 3.—Variations in the vital capacity, expiratory rate, and peripheral blood eosinophils in M. H. following cortisone therapy.

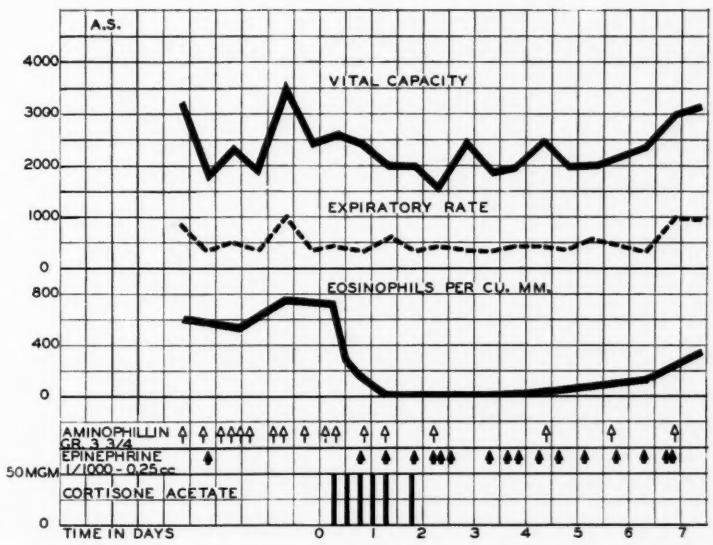

Fig. 4.—Variations in the vital capacity, expiratory rate, and peripheral blood eosinophils in A. S. Although an eosinopenia occurred, this patient's asthma was not influenced as a result of this course of cortisone therapy.

Fig. 3. This patient failed to develop the immediate decrease in eosinophils as ordinarily observed; this may be explained by the fact that she developed local areas of swelling and pain at the site of each injection which may have resulted in a relatively slow absorption of the cortisone. Although eosinopenia subsequently developed, she failed to show any striking change in her asthma after treatment with 300 mg. of cortisone as evidenced by the ventilatory studies and the fact that continued large doses of aminophyllin were necessary for the relief of severe bronchial asthma.

One week after cortisone therapy was started she was given a course of 175 mg. of ACTH intramuscularly in divided doses over a period of 3 days. Improvement in her asthma, rhinitis, and arthritis was noticed on the third day but therapy was discontinued at this time because of edema and a rapid gain in weight. However, she continued to improve clinically for a total period of 2 weeks during which time 10 gr. of aminophyllin and 2 subcutaneous doses of epinephrine daily gave symptomatic relief. Nasal polypi which had been present for years were not visualized on examination. During the following week her asthma gradually recurred in its former severity.

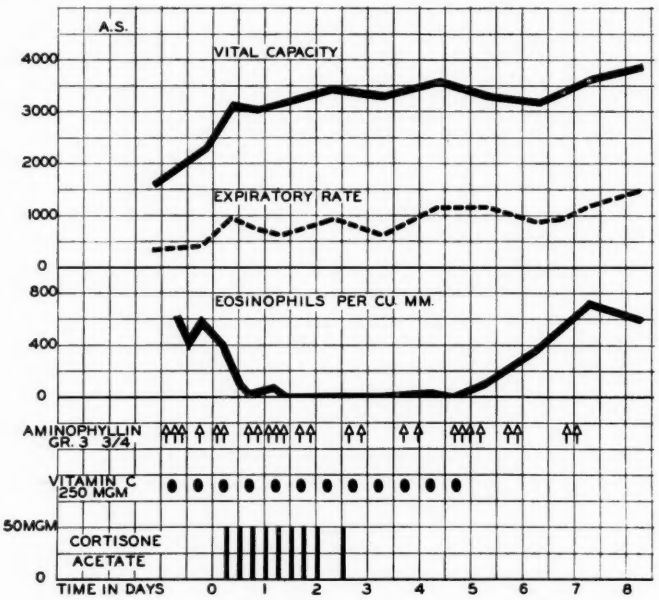

Fig. 5.—Variations in the vital capacity, expiratory rate, and peripheral blood eosinophils in A. S., the same patient as shown in Fig 4. Intravenous vitamin C therapy administered prior to and in combination with cortisone acetate was effective in relieving the asthma in this patient.

CASE 4.—A. S., a 76-year-old retired farmer, had been subject to perennial bronchial asthma since the age of 62 years. Prior to his initial course of therapy with ACTH, he had required large doses of aminophyllin, epinephrine, and oxygen by inhalation several times daily. He also had been subject to hay fever during mid- and late summer for several years and was known to be clinically sensitive to aspirin, ephedrine, and Orthoxine. He had been studied allergically by us in 1940 and again in 1949 and, although known to be sensitive to ragweed, house dust, and several major foods, we had been unable to change the course of his asthma.

On each of 2 previous occasions[5, 8] he was treated with ACTH, which completely relieved his allergic symptoms for periods of 3 weeks.

One week after he had failed to respond to a protracted course of concentrated adrenal cortex extract (Upjohn), previously described,[12] he was started on cortisone acetate (Merck),

receiving 300 mg. in divided intramuscular doses in a period of 2 days. As one may note from Fig. 4, although he developed a prompt eosinopenia, he failed to show any consistent improvement in his asthma as measured by the vital capacity or expiratory rate.

Seven days from the first dose of cortisone, he was given his third course of ACTH consisting of 225 mg. administered intramuscularly over a period of 3 days, with a response camparable to that of the first and second courses previously described in detail.[5, 8]

Six weeks following this course of ACTH, he was readmitted to the hospital having required oxygen and repeated doses of symptomatic medications for the previous 3 weeks. Vitamin C therapy in a daily dose of 250 mg. was administered intravenously for 2 days prior to and during therapy with cortisone acetate. He again showed a similar eosinopenia but in contrast to his previous course of cortisone, he exhibited a prompt improvement in his asthma as judged clinically and as measured by pulmonary ventilatory studies (Fig. 5). This degree of improvement persisted for nearly 3 weeks. It should be noted that this last course of cortisone consisted of 450 mg. as against 300 mg. in the initial course, but it should also be pointed out that he began to improve clinically after the first few injections.

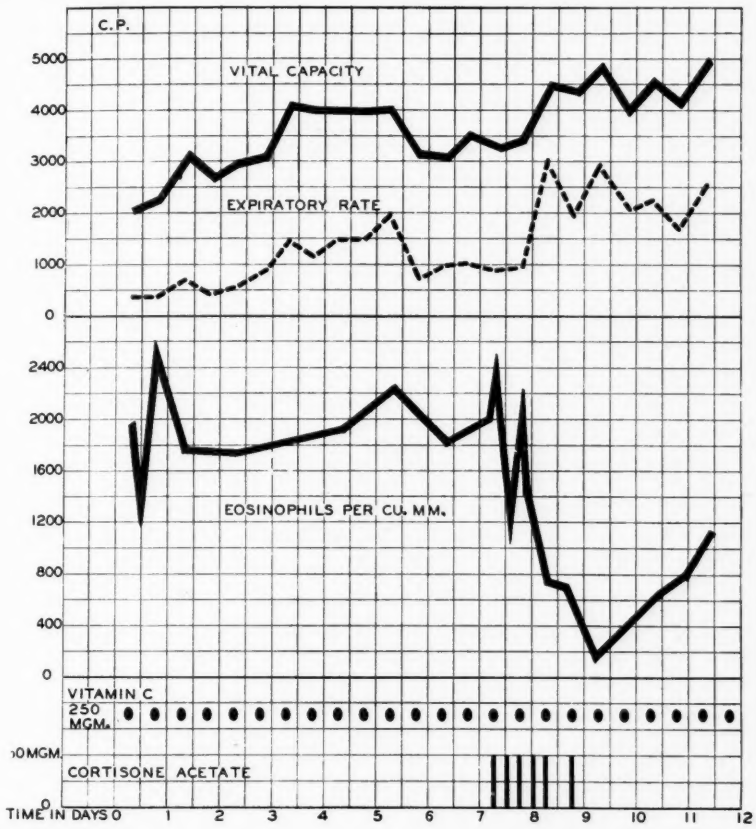

Fig. 6.—Variations in the vital capacity, expiratory rate, and peripheral blood eosinophils in C. P. Oral vitamin C therapy produced a transient improvement in this patient's asthma. Sustained and more complete relief of asthma and rhinitis when cortisone was combined with the oral administration of vitamin C.

Case 5.—C. P., a 43-year-old unemployed man, developed acute sinusitis in the winter of 1942; this was subsequently complicated by asthma. He had had continuous incapacitating bronchial asthma for the past 7 years except for remissions of 1 month following pneumonia and 3 weeks during a self-induced fast. This patient had also been treated

with ACTH on 2 previous occasions[5, 8]; each time he had complete relief of asthma and rhinitis for a period of 3 weeks. Subsequently, treatment with concentrated adrenal cortex extract (Upjohn) failed to change the course of his severe asthma.[12]

In view of the failure of the first course of cortisone therapy in the previous case (A. S.), this patient was pretreated with 250 mg. of ascorbic acid orally every day for 1 week prior to starting and during the administration of cortisone acetate. As may be noted in Fig. 6, there was a material improvement in his asthma as shown by the vital capacity and expiratory rate determinations presumably as a result of vitamin C therapy. This relative improvement persisted only 4 or 5 days and was then followed by a recurrence of moderately severe asthma. At this point and while continuing vitamin C therapy, 300 mg. of cortisone acetate were administered in divided doses over a 48-hour period. His asthma promptly subsided coincident with a decrease in the circulating eosinophils, as illustrated.

The daily oral dose of vitamin C was continued upon discharge from the hospital. Although this combined therapy afforded 75 per cent relief of his asthma for a period of 2 weeks and was of some apparent help for an additional week, it failed to give as complete relief as the 2 previous or 2 subsequent courses of ACTH therapy.

SUMMARY

1. Cortisone acetate in a dosage of 300 mg. administered intramuscularly over a period of 48 hours is relatively effective in the treatment of bronchial asthma.

2. In general, the degree of relief of asthma was not as satisfactory as obtained with 225 mg. of adrenocorticotropic hormone (ACTH) also received by 4 of the 5 individuals of this series as well as several other asthmatics previously reported.[5, 8]

3. One of the latter individuals who failed to show any evidence of sustained improvement in his asthma as a result of his initial course of cortisone acetate showed a reasonably satisfactory clinical response on a second course of cortisone when combined with intravenous vitamin C therapy.

4. A final patient was given vitamin C orally for 1 week; he showed some improvement in his asthma, but subsequently relapsed even though ascorbic acid therapy was continued. However, when cortisone was added to his vitamin C schedule he exhibited a more complete and sustained clinical response.

5. We were prompted to try combined vitamin C and cortisone therapy in these cases since it had been shown by Sayers and Sayers[14] that stimulation of the adrenal cortex in rats was associated with a reduction in the ascorbic acid content of the gland. These observations are of interest in view of the recent work of Lewin and Wassen,[15] showing a cortisone-like effect when ascorbic acid and desoxycorticosterone acetate are administered in combination.

6. Although cortisone is of definite value in the treatment of bronchial asthma, in the doses employed it is less effective than ACTH. Both agents are more effective in uncomplicated cases than in those having other pulmonary pathology.

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THE EFFECT OF ADRENOCORTICOTROPHIC HORMONE (ACTH) ON PATIENTS WITH ALLERGIC DISEASES— FACTS AND SPECULATIONS* † ‡

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PART I

THE AVAILABILITY of a highly purified and potent adrenocorticotrophic hormone (ACTH) has established the fact that not only patients with rheumatoid arthritis and collagen diseases but also patients suffering from certain allergic syndromes respond favorably to its administration.

Studies by Winkenwerder and his co-workers,[1] Randolph,[2] and others have shown that bronchial asthma shows marked improvement 24 to 48 hours after treatment with ACTH is instituted. Our own findings in a series of 6 patients admitted for periods ranging from 32 to 48 days have demonstrated that such improvement is proportional to the metabolic and hematological changes produced by the stimulation of the adrenal cortex. In other words, if a patient fails to show an increase in the urinary excretion of 17-ketosteroids and 11-oxycorticosteroids, a decrease in the number of circulating eosinophils, he also fails to show significant clinical improvement.

METHODS OF STUDY

The studies were carried out in the following manner: Patients suffering from bronchial asthma of comparable severity of either allergic or nonallergic type were admitted, two at a time, to the hospital. They were placed on a diet consisting of basal plus 40 per cent calories; 1 Gm. of protein per kg. body weight with 30 per cent of the calories given as fat; and a daily total of 30 mg. of ascorbic acid. They rested under standard conditions in air-conditioned rooms.

Urinary excretion of 17-ketosteroids was determined by the method of Cahen and Salter.[3] The techniques of Daughaday, Jaffe, and Williams[4] were used for the estimation of 11-oxycorticosteroids and 11-desoxycorticosteroids. A modification of their procedure developed by Calloway, Arrington, and Kyle[5] was employed for the determinations of serum levels of 11-oxycorticosteroids which were carried out at irregular intervals.

Absolute counts of circulating eosinophils were obtained twice daily, at 8:00 A.M. and at noon, throughout the period of admission. We used propylene glycol aqueous stains, described by Randolph.[6]

In order to establish objective standards of clinical improvement, the respiratory capacity of the patients was measured in percentages of the breathing reserve (Tuft and Blumstein[7]). Breathing reserve is a function of maximum voluntary ventilating capacity (M.V.V.C.) and minute volume (M.V.). Breathing reserve (B.R.) $= \frac{\text{M.V.V.C.} - \text{M.V.}}{\text{M.V.V.C.}} \times 100$. The "critical level" of the breathing reserve is reached when it falls to between 70 and 60 per cent; dyspnea develops (Cournand and Richards[8]).

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‡Read at the Sixth Annual Meeting of the American Academy of Allergy, Los Angeles, Calif., March 6-8, 1950.

During the period of observation, the metabolic responses to epinephrine and ACTH* were studied. Eight-tenths milligram of epinephrine was given every 4 hours for 4 consecutive days; 50 mg. of ACTH was given every 4 hours for 4 consecutive days followed by extensive control periods.

RESULTS

Of the 6 patients, 4 recovered a satisfactory respiratory capacity after administration of ACTH; 2 experienced only insignificant changes. The interpretation of the experimental findings is complicated, however, by the lack of data on the metabolic base line in allergic patients.

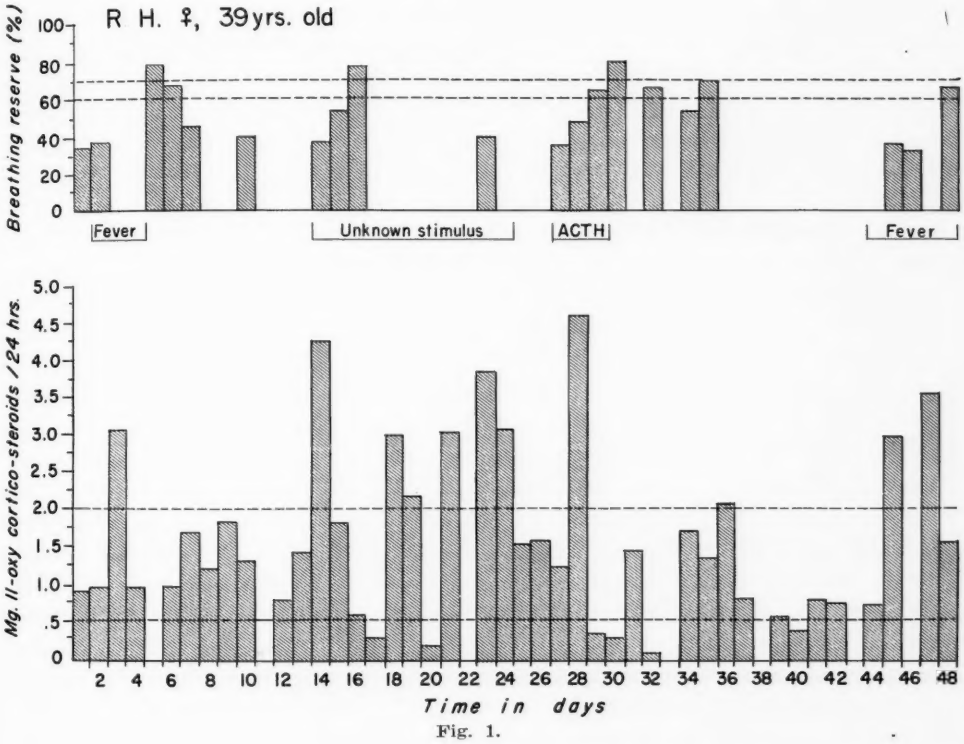

Fig. 1.

Fig. 1 illustrates the variation in the excretion of 11-oxycorticosteroids in a patient suffering from severe bronchial asthma of the nonallergic type, of sudden onset, present for 16 months. The columns of the lower half of the figure indicate the calculated total, in mg./24 hours.

The "normal range" of excretion of 11-oxycorticosteroids extends from 0.5 to 2.0 mg. in 24 hours; these figures are arbitrary and apply only to the particular technique which we have used. The chemical estimation of adrenal corticosteroids is rather crude. It measures the formaldehydogenic substances

*ACTH used in our experiments was supplied by G. D. Searle & Co. They reported "... seven gamma caused a 25 per cent drop in the adrenal ascorbic acid of 26-hour hypophysectomized rats. Armour's standard, by comparison, produced a similar response in 2 quantities. The preparation contained 0.01 U.S.P. units of oxytocin/mg."

of urinary extracts oxidized with periodic acid. It measures, therefore, free cortin; it is likely that a significant amount of adrenal corticosteroids might be excreted in a combined form which escapes determination with methods now commonly in use.

In spite of the limitations of the method, it can be seen, however, that the patient showed marked variations in the excretion of 11-oxycorticosteroids during the period of her admission. On 2 occasions, this variation was evidently due to intercurrent infections which were confirmed by roentgenological evidence and accompanied by total leucocyte counts of 16.000 and 28.000, respectively. Once, increased excretion followed the injection of ACTH, 50 mg. 4 times a day for 3 consecutive days. In addition, we found a persistent increase in the excretion of 11-oxycorticosteroids during a period extending from the fourteenth to the twenty-fourth day of her hospitalization, an increase for which no adequate stimulus could be ascertained. The figure shows a number of other interesting details which we are at a loss to understand, for instance the unexpected decrease in 11-oxycorticosteroid excretion during the third day of ACTH administration and the first day afterwards: we do not feel that the existing evidence would justify the tempting assumption of adrenocortical exhaustion.

The peripheral eosinophil count, which is not included in Fig. 1, varied from 660 to 812 eosinophils per c.mm. at rest. It decreased to 144 and 96, respectively, during the febrile episodes. The unidentified stimulus which accounted for the rise in the excretion of corticosteroids caused a fall in the number of circulating eosinophils without significant diurnal variation during the 10-day period beginning on the fourteenth day of admission. The response of the circulating eosinophils to the administration of ACTH was immediate. Four hours after the initial dose, the absolute count showed a decrease of 78 per cent; eosinophils were absent during the second and third day of the test.

The patient's breathing reserve, expressed in percentages of normal by the columns of the upper half of Fig. 1, reflected adequately the variation in the excretion of 11-oxycorticosteroids. Breathing reserve of the patient on admission was 34 per cent. During the first intercurrent infection, measurements could not be taken; 24 hours after the return of normal temperature, the breathing reserve had increased to 78 per cent. Improvement was of short duration. Each of the subsequent stimuli, however, had a similar effect on the breathing reserve and abolished dyspnea and clinical symptoms of bronchial asthma.

If, as it appears likely, a release of adrenal corticoids after administration of ACTH is responsible for the improvement of allergic manifestations, one might reason that, in turn, bronchial asthma might be due to: (1) failure of the pituitary to stimulate the adrenal cortex; (2) failure of the adrenal cortex to respond to pituitary stimulation, or (3) as recently suggested by Godlowski[9] in studies on rheumatoid arthritis, to a lowered sensitivity of the mesenchymal tissue to physiological concentrations of adrenocortical hormones. The first two possibilities lend themselves to clinical investigation. Since the patient whose record we have presented responded to the administration of ACTH with increased excretion of corticosteroids and with clinical relief, it is conceivable that her bronchial asthma is caused by the inability of the pituitary to stimulate the adrenal cortex sufficiently or at all times. Patients who are refractory to treat-

ment with ACTH, on the other hand, might have a functional deficiency of at least part of the adrenal cortex which prevents the release of certain corticoids in spite of proper pituitary stimulation.

We believe that one of our recent findings might support the latter hypothesis. We have been able to produce attacks of bronchial asthma in allergic individuals during symptom-free intervals by prolonged administration of epinephrine hydrochloride (0.8 mg., subcutaneously, every 4 hours, for 48 hours). The evidence is not conclusive since Hamburger, Milliez, and Halpern[10] have demonstrated that epinephrine might cause secondary bronchoconstriction as the result of an "autonomic rebound." It is suggestive, however, of the possibility that continuous stimulation might exhaust the adrenocortical reservoir. We have undertaken a comparative study of serum levels of 11-oxycorticosteroids of epinephrine-fast and epinephrine-responsive patients in the hope of adding to the understanding of epinephrine fastness. So far, the number of patients under observation is too small to permit even tentative conclusions.

The third possibility suggests the shock tissue itself as the site of altered function. In discussing it, we must re-examine our concepts of the pathogenesis of bronchial asthma.

Part II

The principal physiological mechanisms which are thought to enter the etiology of allergic manifestations are illustrated in Fig. 2. Until the advent of ACTH, attention has been focused on two potential pathways: (1) the liberation of histamine which is the result of antigen-antibody reactions of the anaphylactic type, and (2) an "imbalance" of the autonomic nervous system at which parasympathetic stimuli predominate. The first concept implies that histamine released through enzymatic action from tissue fixed into free state causes smooth muscle contraction, increased capillary permeability, and secretion of mucous glands. The second concept, originally proposed by Eppinger and Hess,[11] suggests that the same syndrome can be produced by selective stimulation of the vagus nerve.

The first concept stands accepted, while much debate has centered around the second. Most authors were reluctant to agree with a hypothesis which was, essentially, based on controversial animal experiments. Interest has been renewed, however, since European workers[12] have shown that patients with chronic bronchial asthma exhibit severe degenerative changes in the sympathetic chain; they suggest that the impaired sympathetic nerves might be unable to balance a hyperactive or even a normally active parasympathetic system. Scudamore, Vorhaus, and Kark,[13] on the other hand, made observations on the cholinesterase activity of serum from patients ill with asthma and measured acetylcholine in their blood. In a personal communication, the authors state: "Our observations show, in blood from asthmatics, normal levels of cholinesterase activity and high levels of acetylcholine. In one patient who was in status asthmaticus when the blood was drawn, the acetylcholine level was extremely high. These results could mean that in asthmatics there is an overproduction of acetylcholine, or an interference with the normal mechanism of acetylcholine breakdown. They do not tell us whether the high acetylcholine levels are the

cause or the result of asthma. They offer a reasonable explanation for the clinical observation that morphine is a dangerous drug to use in the treatment of patients ill with asthma.''

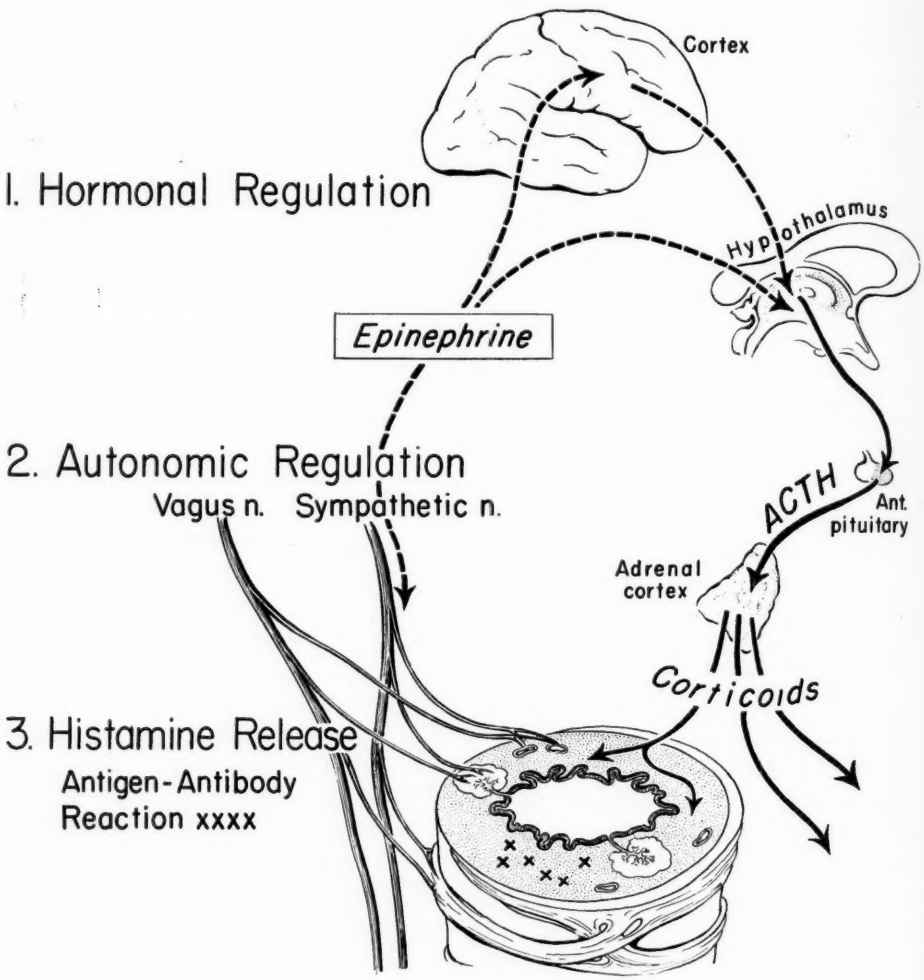

Fig. 2.

The effects of adrenocorticotrophic hormone and of adrenal corticoids on patients ill with allergic diseases have added a third concept to the former two, a concept which adds a ubiquitous shock tissue, the mesenchyme, to those already known and discussed. The mode of action of the adrenal hormones on the target organ, the connective tissue, is not known; altered permeability and interference with enzyme systems have been mentioned (Thorn and Bayles[14]).

Gersh and Catchpole[15] have proposed an interesting theory in an attempt to explain changes in ground substance and basement membranes. Ground sub-

stance, a mucopolysaccharide, exists under normal conditions in an inert, polymerized form. Enzymatic depolymerization caused by injury, inflammation, hormonal stimulation, and other factors can alter its structure and render it water soluble. Thus "... depolymerization of glycoprotein of the ground substance has certain implications in a general consideration of the 'permeability' of blood vessels. In the first place, the change in physical consistency must influence the diffusion of nutrients and metabolites between cells and blood vessels. In the second place, the process of depolymerization must result in the exposure of an increased number of reactive groups. Thus the relatively inert glycoprotein of ground substance is converted to substances which may react with some of the diffusing constituents. The removal of such substances through chemical combination should cause an increased passage of materials from blood vessels." In spite of the absence of experimental evidence, it seems reasonable to assume that a similar mechanism exists in the connective tissue of the bronchial tree.

While it is possible to consider the 3 mechanisms which are active in the etiology of bronchial asthma as separate entities, we are aware of the fact that they are bridged by various links. The most obvious bond is formed by the dual action of epinephrine which, on one hand, controls the sympathetic discharges, and on the other, initiates through stimulation of the hypothalamus the chain of hormonal reactions which results ultimately in the release of adrenal corticoids. Other linkages are present: Dragstedt, Mills, and Mead[16] demonstrated that administration of cortical hormone does not prevent anaphylactic shock, but increases the resistance of animals to the sequels of the antigen-antibody reactions. Moreover, the level of histamine in the blood of animals subsequent to anaphylactic shock was as high in "protected" as in unprotected animals. Dragstedt's findings are compatible with those of others who found that, conversely, adrenalectomy increases the sensitivity of experimental animals to sensitization and reinjection of specific antigens (Flashman[17]).

In recent months, we made use of an old observation to investigate a possible relationship between tissue permeability and administration of adrenocorticotrophic hormone. It had been shown[18] that patients suffering from bronchial asthma have a diminished tolerance to the inhalation of aqueous solutions of histamine diphosphate; in other words, they develop attacks of bronchial asthma after inhalation of dilutions which fail to cause symptoms in nonallergic individuals. In the light of the concepts which we have discussed, it seemed worth while to repeat the studies before and after injection of ACTH. It was found that the increase in breathing reserve is accompanied by an increase in tolerance to histamine, a finding which is compatible with, but not conclusive for, the assumption that the permeability of the barrier, the connective tissue, has changed.

The evidence which we have presented suggests that ACTH through the release of adrenal corticosteroids alters the mesenchyme of the bronchial shock tissue in a manner which makes it less vulnerable to specific and nonspecific agents. ACTH acts equally well on allergic and nonallergic bronchial asthma if the adrenals are intact and responsive. On the other hand, we feel that deficient, possibly faulty function of anterior pituitary or adrenal cortex can originate

the nonallergic type of bronchial asthma, and perpetuate the allergic type. The recognition that fever is able to correct certain allergic conditions has convinced us that ACTH and adrenal corticoids are effective by restoring a disturbed physiological equilibrium rather than by a druglike action as has been suggested. The data, which have been collected on the effectiveness of ACTH in various diseases, emphasize the nonspecific nature of its action; it is conceivable, however, that an intimate relationship exists between adrenal corticosteroids and enzyme systems involved in the mechanism of the allergic reaction. Furthermore, while we are aware of the link between sympathetic stimuli and hormonal regulations, a similar link might connect parasympathetic centers and pituitary although it has, so far, escaped our attention.

ACTH controls the clinical symptoms of allergic rhinitis, bronchial asthma, urticaria, and atopic dermatitis. After withdrawal of the hormone, the symptoms recur inevitably in the same shock tissue. Accordingly, we submit that additional factors independent of pituitary-adrenal regulations determine the site of the shock reaction in the allergic individual. The investigation of these factors will be an integral part of further studies on the role of adrenocorticotrophic hormone and adrenal corticoids in the course of the diseases of allergy.

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THE EFFECT OF PITUITARY ADRENOCORTICOTROPHIC HORMONE (ACTH) ON HISTAMINE INTOXICATION AND ANAPHYLAXIS IN THE GUINEA PIG*†

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CLINICAL observations have recently been made concerning the beneficial effects of pituitary adrenocorticotrophic hormone (ACTH) and the adrenal steroid, 11-dehydro-17-hydroxycorticosterone-21-acetate (cortisone) in a number of conditions where hypersensitivity is considered to be an important etiological factor. Rheumatoid arthritis, rheumatic fever, disseminated lupus erythematosus, as well as common allergic syndromes such as asthma, hay fever, urticaria, and eczema are reported to be benefited by the administration of these hormones.[1-5] These observations have stimulated increased interest in the relationship of the pituitary-adrenal system to allergic disease. Two avenues of investigation which may be considered in attempting to elucidate this problem are the influence of the pituitary and adrenal secretions on antibody formation and their effect on antigen-antibody union. The present study concerns itself with the influence of ACTH on antigen-antibody union in the guinea pig. Since there is strong evidence that histamine is released during anaphylaxis in this animal species,[6] two phases of the problem are considered. First, the effect of ACTH on histamine intoxication, and second, the influence of ACTH on the anaphylactic reaction itself.

A brief review of studies carried out by previous investigators in this field may be cited as a background for the present experiments. Much of the past work concerning the relationship of the adrenals to these phenomena has been carried out in the rat or mouse. It is well known that the normal rat is quite resistant to histamine poisoning and to anaphylactic shock. Adrenalectomy, however, renders the rat more susceptible to the toxic effects of histamine,[7] and it has also been reported that anaphylaxis is more readily induced in such animals.[8] The lethal intraperitoneal dose of histamine phosphate for normal albino rats is more than 100 mg. per 100 grams of body weight, while in the adrenalectomized rat, according to Wyman,[9] the lethal dose is approximately 7 mg. per 100 grams. The administration of cortin was found by Gottesman and Perla to restore the resistance of such animals to histamine.[10] The histaminase content of rat tissue is reported by Karady, Rose, and Browne,[11] to decrease after adrenalectomy with a consequent reduction in the rate of histamine inactivation.[12] It has been further noted by Rose[13] that either adrenal cortical extract or desoxycorticosterone are capable of restoring the ability of the adrenalectomized rat to inactivate histamine. More recently, Selye[14] noted the protective action of cortisone in adrenalectomized rats against the "anaphylactoid" reaction occurring from a single injection of egg white. Desoxy-

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corticosterone on the other hand was found to enhance this reaction. ACTH was observed to protect, while anterior pituitary extract appeared to aggravate this response in intact rats. This "anaphylactoid" reaction to egg white demonstrated in rats may be prevented by prior treatment with antihistaminic drugs,[15] suggesting that a histamine-release mechanism is involved.

The guinea pig has been used less frequently in the study of this problem, perhaps because adrenalectomy is more difficult to perform. In contrast to the rat, however, the guinea pig is extremely susceptible to the lethal effects of histamine. Approximately 0.3 mg. per kg. of histamine base intravenously is a 100 per cent lethal dose.[16] The guinea pig is also an excellent subject for the study of anaphylaxis, since anaphylactic sensitization is readily achieved. The adrenal as a factor in guinea pig anaphylaxis was studied by Kepinov,[17] who reported that smaller doses of antigen were required to induce fatal shock in adrenalectomized pigs than in normal animals. Wolfram and Zwemer[18] found that cortin given 2 to 6 hours prior to the shocking dose of antigen protected guinea pigs against anaphylaxis. More recently, Leger, Leith, and Rose[19] observed no protection against anaphylaxis in guinea pigs receiving ACTH. In the dog, Dragstedt, Mills, and Mead[20] found that prior administration of adrenal cortex extract to sensitized animals diminished the severity, but did not prevent acute anaphylactic shock. The blood levels of histamine during anaphylaxis in treated and untreated animals were comparable.

MATERIALS AND METHODS

The preparation of ACTH used was Lot 77-S, having a potency of 41 per cent of Armour standard La-I-A, and containing 0.002 oxytocic units per milligram.* All doses mentioned in this study are recorded in terms of the Armour standard. Solutions were freshly prepared in saline before use, and administered subcutaneously.

Histamine Intoxication.—The aerosol chamber technique was used in these experiments. It has been shown by Mayer[21] that exposure of guinea pigs to histamine aerosols which are nonfatal do not significantly alter tolerance to subsequent exposures. Thus, it is possible to test the same animal repeatedly, if an end point short of fatal shock is selected, and a sufficient period of time is allowed to elapse between exposures for the guinea pig to recover. The apparatus, similar to that described by Loew, Kaiser, and Moore,[22] consisted of a cylindrical glass jar, 4.5 gallon capacity, inverted over a desiccator cover. A glass nebulizer fixed in the center of the cover was connected to a compressed air line with a mercury manometer interposed. A solution of histamine phosphate containing 0.25 mg. of histamine base per c.c. was freshly prepared, and 1 c.c. of this material aerosolized into the chamber within 15 to 20 seconds when an air pressure of 150 mm. Hg was applied.

Male guinea pigs, weighing from 200 to 225 grams, were used. The animal in each instance was placed in the chamber, and 1 c.c. of histamine solution aerosolized during the first 15 to 20 seconds. The guinea pig was allowed to remain until the first sign of coughing or choking occurred. Utilizing this symp-

*The ACTH used in this study was supplied by the Department of Biochemical Research, The Armour Laboratories, Chicago, Ill.

tom as an end point, the animal was removed from the chamber and the exposure time recorded. Repeated trials were carried out in each animal over a period of 6 hours. Nine animals who received 1.64 to 4.1 mg. of ACTH were tested before treatment, and at intervals of 2, 4, and 6 hours after ACTH was injected. These results were compared with the tolerance time displayed by 5 untreated animals at similar exposure intervals.

Anaphylaxis.—Thirty-five male guinea pigs, 200 to 250 grams in weight were actively sensitized by the intraperitoneal injection of 0.10 c.c. of horse serum. Fourteen to 18 days later, a second dose of horse serum was injected intravenously through one of the penile veins. Twenty animals were given 1.64 to 4.1 mg. of ACTH subcutaneously 4 hours before the second dose of antigen. Fifteen remaining animals received no prior treatment. The shocking dose of horse serum employed was varied from 0.50 c.c. of the normal serum to 0.10 c.c. of a 1 to 10 dilution of the same serum (0.5 c.c. to 0.01 c.c.) to determine, if possible, the minimal shocking dose in treated and control pigs. Results were graded as fatal shock, severe shock (convulsions, collapse, coma, with recovery), moderate shock (extreme dyspnea, coughing, choking), mild shock (rubbing of nose, sneezing, ruffling of hair), and no shock.

RESULTS

Histamine Intoxication.—Guinea pigs treated with ACTH failed to exhibit any marked difference in their response to aerosolized histamine before and at intervals after ACTH (Table I). Most animals developed symptoms within 3 minutes, and in no instance were able to remain in the chamber beyond 5½ minutes without manifestations of severe choking dyspnea, coughing, or convulsions. While some increase or decrease in tolerance time occurred before and after ACTH treatment, the variations observed were not greatly different from those occurring in untreated animals upon successive exposures to his-

TABLE I. EFFECT OF ACTH ON HISTAMINE INTOXICATION IN THE GUINEA PIG
(Length of time in aerosol chamber before appearance of bronchospasm)

ANIMAL NUMBER	TIME IN CHAMBER BEFORE ACTH	DOSE OF ACTH MG. OF ARMOUR STANDARD	TIME IN CHAMBER HOURS AFTER ACTH		
			2 HOURS	4 HOURS	6 HOURS
1	2′ 0″	0	1′ 40″	1′ 20″	1′ 30″
2	2′ 15″	0	2′ 30″	2′ 0″	1′ 30″
3	3′ 55″	0	4′ 15″	4′ 0″	3′ 10″
4	1′ 45″	0	1′ 40″	2′ 0″	2′ 15″
5	1′ 0″	0	2′ 50″	1′ 10″	1′ 50″
6	2′ 20″	1.64	1′ 20″	1′ 30″	1′ 20″
7	2′ 0″	1.64	2′ 30″	2′ 30″	3′ 15″
8	1′ 15″	1.64	90″	1′ 20″	3′ 0″
9	1′ 10″	2.46	1′ 0″	1′ 20″	2′ 15″
10	4′ 30″	2.46	3′ 0″	3′ 05″	3′ 50″
11	3′ 0″	2.46	2′ 30″	2′ 15″	3′ 0″
12	4′ 0″	4.1	4′ 30″	5′ 10″	4′ 40″
13	2′ 0″	4.1	2′ 40″	2′ 0″	2′ 15″

′ = minutes
″ = seconds

One c.c. of histamine solution containing 0.25 mg. of base per c.c. aerosolized into chamber during first 15 to 20 seconds.

tamine under the same conditions. In contrast to this lack of protection, similar guinea pigs treated with 3 mg. per kg. of Neoantergan (Merck) subcutaneously 15 minutes before exposure in the chamber were able to tolerate the aerosolized histamine atmosphere for at least 10 minutes without evidence of dyspnea.

Anaphylaxis.—Sensitized guinea pigs treated with ACTH 4 hours before an intravenous shocking dose of horse serum exhibited no apparent resistance to anaphylaxis (Table II). Shock of varying degree was observed in all treated and control animals. Fatal anaphylaxis occurred in 11 of 20 ACTH treated guinea pigs (a mortality of 55 per cent) as compared to 8 deaths in 15 control animals (a mortality of 53.3 per cent). The degree of shock varied in most cases directly with the amount of antigen injected intravenously (Table III). A dose of 0.10 c.c. to 0.50 c.c. of normal horse serum resulted in fatal shock in 6 of 7 treated animals, and in 4 of 4 controls. Smaller amounts of horse serum, 0.01 c.c. to 0.05 c.c., caused death in 5 of 13 ACTH treated guinea pigs, and in 4 of 11 untreated animals.

TABLE II. ANAPHYLAXIS GUINEA PIGS

(Animals sensitized with 0.10 c.c. horse serum intraperitoneally. Shocking dose of horse serum administered intravenously 14 to 18 days later. ACTH injected subcutaneously 4 hours prior to shocking dose of antigen)

GUINEA PIG NUMBER	AMOUNT OF ACTH MG. OF ARMOUR STANDARD	DOSE OF HORSE SERUM I.V. C.C.	DEGREE OF SHOCK
1	0	0.50	fatal
2	0	0.25	fatal
3	0	0.12	fatal
4	0	0.10	fatal
5	0	0.05	fatal
6	0	0.05	moderate
7	0	0.025	fatal
8	0	0.025	fatal
9	0	0.025	mild
10	0	0.025	severe
11	0	0.025	mild
12	0	0.01	moderate
13	0	0.01	moderate
14	0	0.01	fatal
15	0	0.01	mild
16	1.64	0.50	fatal
17	1.64	0.25	fatal
18	2.46	0.25	fatal
19	2.46	0.10	fatal
20	2.46	0.05	mild
21	2.46	0.05	severe
22	2.46	0.05	fatal
23	2.46	0.05	fatal
24	2.46	0.025	fatal
25	2.46	0.025	mild
26	2.46	0.025	severe
27	2.46	0.01	mild
28	2.46	0.01	mild
29	2.46	0.01	mild
30	4.1	0.10	mild
31	4.1	0.10	fatal
32	4.1	0.10	fatal
33	4.1	0.05	fatal
34	4.1	0.025	moderate
35	4.1	0.025	fatal

TABLE III. ANAPHYLACTIC SHOCK. RELATION OF DOSE OF ANTIGEN TO FATAL SHOCK

AMOUNT OF HORSE SERUM I.V. C.C.	CONTROL		ACTH TREATED					
			1.64 MG.		2.46 MG.		4.1 MG.	
	TOTAL USED	NO. FATAL	TOTAL USED	NO. FATAL	TOTAL USED	NO. FATAL	TOTAL USED	NO. FATAL
0.10 to 0.50	4	4	2	2	2	2	3	2
0.05	2	1	–	–	4	2	1	1
0.025	5	2	–	–	3	1	2	1
0.01	4	1	–	–	3	0	–	–
Total	15	8	2	2	12	5	6	4

DISCUSSION

Under the conditions described in this study, the doses of ACTH employed failed to alter the sensitized guinea pig's normal response to a second dose of specific antigen. ACTH did not prevent the toxic effects of comparatively small amounts of exogenous histamine in the normal guinea pig. This would suggest that the increased secretion of cortical steroid stimulated by the administration of such amounts of ACTH does not affect the union of antigen with antibody in vivo, or prevent the liberation of histamine resulting from such union. It is realized that increased dosage and prolonged treatment with ACTH, as well as many other variations which were not explored in the study because of limitations in the supply of ACTH, may perhaps disclose an effect not apparent in the present experiments. However, Fischel[23] in a recent paper mentions that ACTH had no effect in the tissue damage resulting from the union of antigen and antibody in the Arthus and anaphylaxis reactions studied by passive, quantitative methods.

The possible influence of the pituitary-adrenal system on the formation of antibody had also been studied by a number of workers. Dougherty, White, and Chase[24-27] observed that injections of adrenal cortical steroid or their increased secretion as the result of ACTH injection caused a rise in antibody titers in sensitized animals. Fischel, LeMay, and Kabat,[28] as well as Thatcher, Houghton and Ziegler,[29] on the other hand, were unable to produce an anamnestic response in animals by the injection of ACTH. Eisen and his collaborators[30] found identical concentrations of serum antibodies and gamma globulins in adrenalectomized rats receiving adrenal cortical extract during immunization and in similar animals not receiving cortical extract. In normal human subjects, Herbert and DeVries[31] reported that circulating antibody levels were not increased following administration of large quantities of ACTH. During the treatment of patients with rheumatoid arthritis, Ragan, Grokoest, and Boots[32] observed that agglutinin titers for Group A hemolytic streptococci and for sensitized sheep cells were unchanged by ACTH.

From the experiments in the present study, together with the rather wide variance in the observations of a number of workers herein cited, there appears to remain some question concerning the manner in which the pituitary-adrenal system influences the immune mechanism.

SUMMARY

1. Observations were made on the effect of pituitary adrenocorticotrophic hormone (ACTH) on histamine intoxication and anaphylaxis in the guinea pig.

2. The time required for guinea pigs to develop bronchospasm when exposed to aerosolized histamine was approximately the same before or after the administration of a single dose of ACTH.

3. Guinea pigs actively sensitized to horse serum 14 to 18 days previously, and who were given ACTH 4 hours before the second dose of horse serum, developed anaphylactic reactions comparable to those displayed by sensitized controls who had received no prior treatment with ACTH.

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THE EFFECT OF CORTISONE UPON THE RELEASE OF HISTAMINE DURING IN VITRO HEMOLYTIC REACTIONS IN RABBIT BLOOD*†

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INTRODUCTION

IT HAS been demonstrated in vitro that histamine is released into the plasma of the blood of rabbits and human beings sensitive to such antigens as egg albumin and ragweed pollen when these are added to the blood.[1-5] A similar release of histamine accompanied in vitro hemolytic reactions in the blood of appropriately sensitized rabbits.[6] In recent years it has been shown that compounds from the adrenal cortex may influence the concentration of antibodies in the blood by liberating them from cells.[7, 8] Such an effect might conceivably alter the reaction of sensitized cells. This study was undertaken to determine whether or not the addition of cortisone to blood of rabbits sensitized to sheep erythrocytes would affect the release of histamine which uniformly accompanies the hemolytic reaction produced by mixing the blood and the sheep cells.

METHODS

Rabbits were used throughout the study. They were sensitized to washed sheep erythrocytes, and the hemolysin titer subsequently was determined according to modifications of techniques reported by Kolmer.[9] Blood was collected from the sensitized rabbits in paraffin-coated containers from the cut end of the carotid artery, care being taken to prevent trauma to the formed elements of the blood. Determinations of the histamine content of the blood or plasma were made according to the Code modification[10] of the Barsoum and Gaddum procedure.

All additions of cortisone to blood were made from a stock physiologic solution of sodium chloride containing 100 μg. of cortisone per cubic centimeter.‡

When testing the effect of cortisone on the release of histamine occurring during in vitro hemolytic reactions, 4 sets of samples of blood were prepared. To one set serving as a control, physiologic salt solution was added; to another, cortisone; to the third, sheep erythrocytes in physiologic salt solution, and to the fourth, cortisone and sheep erythrocytes in physiologic salt solution. The amounts added in each instance were such that the final volumes of all of the samples were equal. All were then incubated simultaneously for one hour at 37° C. After incubation, the samples were centrifuged and samples of the plasma mixtures withdrawn for histamine determinations. When sufficient blood was available, tests were carried out in duplicate or triplicate.

During the study, the following control observations were made. Tests were made of the effect of cortisone on the contraction of the isolated ileum of the guinea pig in response to histamine. Tests were also made for any possible

*From the Division of Medicine and Section on Physiology, Mayo Foundation and Mayo Clinic.

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‡The cortisone used in these experiments was kindly given to us by Dr. H. L. Mason.

effect which cortisone might have on histamine in simple solution. The effects of in vitro additions of cortisone on the distribution of histamine in the blood and on the destruction of histamine known to occur in blood were also determined.

RESULTS

The Effect of Cortisone on the Release of Histamine During In Vitro Hemolytic Reactions.—The presence of 10 to 50 μg. of cortisone per c.c. of blood did not affect the increase in the histamine content of the plasma which always accompanied the occurrence of hemolytic reactions in the blood (Table I). Since it was possible in these tests that cortisone had not had sufficient time to act upon the cells, the hormone was placed in samples of blood and they were then incubated for 1 hour before the sheep erythrocytes were added. The additional time for cortisone action did not affect the results; the hormone still had no effect on the release of histamine by the hemolytic reaction.

Control Observations.—The presence of 10 μg. of cortisone per c.c. in the solution surrounding the guinea pig ileum for periods of 1 minute did not affect subsequent contractions of the intestine to histamine. The incubation of solutions containing 0.1 μg. of histamine per c.c. with cortisone in concentrations varying from 1 to 50 μg. per c.c., for periods ranging from one-half to 3 hours had no effect upon the amount of histamine present in the solutions as determined by contractions of the ileum of the guinea pig.

TABLE I. THE EFFECT OF INCUBATION OF RABBIT BLOOD FOR ONE HOUR WITH CORTISONE UPON HISTAMINE RELEASE INTO PLASMA IN COURSE OF HEMOLYTIC REACTIONS

RABBIT	HEMOLYSIN TITER OF BLOOD	MICROGRAMS OF CORTISONE PER C.C. OF BLOOD	MICROGRAMS OF HISTAMINE PER C.C. OF BLOOD PLASMA			
			NO HEMOLYSIS		HEMOLYSIS	
			NO CORTISONE	WITH CORTISONE	NO CORTISONE	WITH CORTISONE
1. (A)	*	10	0.32	0.46	0.60	0.32
2. (C)	*	10	0.67	0.71	1.28	0.75
3. (502)	1:40,960	10	0.75 0.71		1.20	1.00‡ 1.09‡
4. (503)	1:20,480	10	0.25 0.09		1.25	1.00‡ 1.25‡ 0.87‡
5. (47)†	1: 2,560	50	0.13 0.13	0.12 0.13	0.28	0.17 0.17
6. (91)†	1: 5,120	10	0.07 0.07		0.80 0.70	0.76‡ 0.80
7. (93)†	1:10,240	10	0.09 0.08		1.14 1.22 1.33	1.00 1.00
8. (498)†	1:10,240	10	0.20 0.33		1.25 1.41	1.67‡ 1.56‡

*Extensive hemolysis in plasma of hemolytic system.
†Paraffin-coated glass technique employed.
‡Sheep erythrocytes added following 30 minutes' incubation of whole blood with cortisone.

Incubation of blood from 2 normal rabbits with cortisone in amounts sufficient to make a final concentration of 50 μg. per c.c. had no effect upon the histamine content of the blood. The concentration of histamine in the blood of

the 2 animals before incubation was 2.6 and 2.1 μg. per c.c. After 4 hours of incubation of the blood with physiologic salt solution, the values were 2.3 and 1.6 μg. per c.c., respectively. The amounts of histamine present in the samples incubated with cortisone were the same. The disappearance of histamine which accompanied the incubation was therefore unaffected by the presence of cortisone.

In another series of experiments, the effect of incubating whole blood with cortisone upon the histamine content of the plasma was determined. This was done to check the possibility that the presence of cortisone might produce changes in the distribution of histamine in the blood. In these tests, the final concentration of cortisone in the blood ranged from 10 to 50 μg. per c.c. and the time of incubation ranged from 1 to 4 hours. After incubation, the blood was immediately centrifuged and plasma samples taken for histamine determination. In the early experiments no special care was taken to prevent trauma to the blood, and paraffin tubes were not used. The concentration of histamine in the plasma was high in these tests. In 2 instances, care was taken to prevent trauma in drawing the blood, and the tubes used to collect the blood were coated with paraffin. In these, the histamine content was low. In neither of the groups of experiments was there any consistent effect of cortisone upon the histamine content of the plasma (Table II).

TABLE II. EFFECT OF INCUBATION OF WHOLE BLOOD WITH CORTISONE ON HISTAMINE CONTENT OF PLASMA

RABBIT	HOURS OF INCUBATION	MICROGRAMS OF CORTISONE PER C.C. OF BLOOD	MICROGRAMS OF HISTAMINE PER C.C. OF PLASMA MIXTURE FROM WHOLE BLOOD TO WHICH HAD BEEN ADDED :	
			CORTISONE	0.9 PER CENT SALT SOLUTION
A	1	25	0.43	0.67
B	1	25	0.50	0.71
C	1	10	0.46	0.32
D	1	10	0.71	0.67
E	4	50	0.57	0.44
F	4	50	1.45	0.57
G	4	50	0.94	0.36
H	4	50	0.30	0.48
I	4	50	0.17	0.24
J	4	50	0.48	0.51
K*	4	50	0.12 0.14	0.11 0.11
L*	1	50	0.12 0.13	0.13 0.13

*Paraffin-coated equipment employed; duplicate experiments performed.

COMMENT

In this study additions of cortisone to blood of rabbits had no effect upon the release of histamine into the plasma which uniformly accompanies a hemolytic reaction. Additions of the hormone also had no effect upon the histamine content of whole blood, the distribution of the histamine in the blood as determined by its concentration in the plasma, and no effect upon the disappearance

of the histamine from the blood during incubation. All of the studies were carried out in vitro, the cortisone being added to drawn blood and never administered to the intact animal. The results might have been quite different if the hormone had acted upon the intact organism or upon other tissues. At the time these tests were made, the hormone was not available in sufficient quantities for adequate in vivo experiments.

SUMMARY

1. The in vitro addition of cortisone to the blood of rabbits previously sensitized to sheep erythrocytes had no effect upon the release of histamine into the plasma which uniformly accompanied the hemolytic reaction produced by mixing the blood with the sheep cells.

2. Incubation of the blood with the cortisone for 1 hour prior to addition of the sheep cells did not alter the result.

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THE EFFECT OF TRIPELENNAMINE HYDROCHLORIDE (PYRIBENZAMINE) ON THE RELEASE OF HISTAMINE DURING IN VITRO HEMOLYTIC REACTION IN RABBIT BLOOD*†

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IT HAS been shown by in vitro studies of anaphylactic reactions that histamine is released into the blood plasma of rabbits and human beings sensitized to egg albumin, ragweed pollen, or other foreign substances, when the appropriate sensitizing agent is added to the blood.[1-5] Recently, a similar liberation of histamine was found to accompany in vitro hemolytic reactions in rabbit blood.[6] The histamine content of blood plasma of rabbits sensitized to sheep erythrocytes was increased from 2 to 15 times during an in vitro hemolytic reaction. The present investigation was undertaken to determine the effect of an antihistaminic agent, tripelennamine hydrochloride, on the release of histamine in the course of in vitro hemolytic reactions. Tripelennamine hydrochloride (hereinafter referred to as pyribenzamine‡ has been shown to reduce or eliminate anaphylactic reactions in a number of different species of animals.[7-11] The mechanism by which this reduction takes place is not established, although most investigators regard the effect as due to blocking of the action of histamine by the antihistaminic agent at the various sites of histaminic action.[12] The experiments to be reported were specifically designed to determine whether or not pyribenzamine would prevent or alter the release of histamine into the plasma during a hemolytic reaction.

In order to assay the histamine in samples of blood to which pyribenzamine had been added, it was necessary to remove the drug from the blood. To obtain some measure of how complete this removal had to be, tests were made on the terminal ileum of the guinea pig to determine the smallest amounts of pyribenzamine which would interfere with the action of histamine.

METHODS

Rabbits were sensitized to sheep erythrocytes and samples of blood were obtained according to technique previously described.[6] Determinations of histamine were made with the use of Code's modification of the Barsoum and Gaddum method.[13] When pyribenzamine had been added to the blood an additional procedure was necessary to remove it from the samples. This was accomplished by taking advantage of the solubility of pyribenzamine in ether. The final extracts were shaken in separating funnels for 5 minutes with equal volumes of ether. The ether was then discarded and the procedure repeated twice. Any ether which remained after the third separation was removed by suction with the sample immersed in a bath of warm water. It was found that quantities of pyribenzamine added to make concentrations as high as 20 μg per ml.

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†From the Division of Medicine and Section of Physiology, Mayo Foundation and Mayo Clinic.

‡The pyribenzamine used in this study was kindly provided by Dr. E. Oppenheimer, Ciba Pharmaceutical Products, Inc., Summit, N. J.

of blood could be completely removed from the extracts of the plasma by this procedure. Removal of both pyribenzamine and ether had to be complete because minute quantities of either substance made assay of the extracts on the ileum of the guinea pig impossible.

When an experiment was carried out, 10 ml. samples of blood were used. To these either physiologic salt solution or pyribenzamine in physiologic salt solution was added in amounts sufficient to make a final concentration of 1 μg. of pyribenzamine per ml. of blood. The samples were then incubated at 37° C. for one-half hour. Immediately after incubation, 0.5 ml. of a 50 per cent suspension of washed sheep erythrocytes in physiologic saline solution was added to one-half of the samples containing physiologic salt solution and to all of those containing pyribenzamine. A similar volume of physiologic saline solution was placed in those samples to which the erythrocytes had not been added to make the final volume of all samples equal. Incubation was then continued for an additional half hour; the samples were centrifuged and samples of plasma were taken to determine the amount of histamine contained in each.

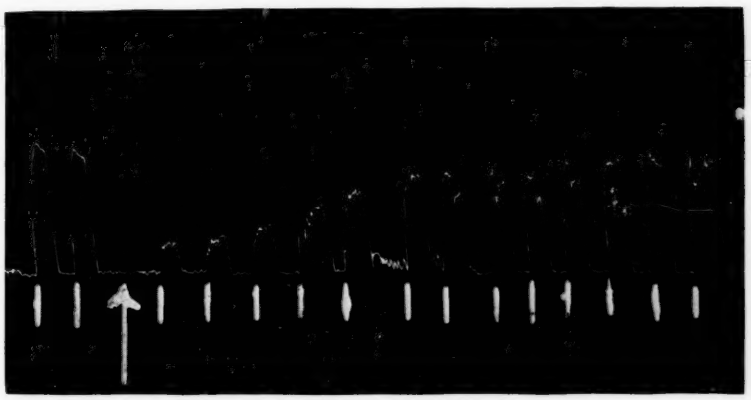

Fig. 1.—The effect of pyribenzamine on the contractions produced by histamine in a strip of isolated ileum of a guinea pig. Pyribenzamine in a dilution of 1:1,000,000,000 was in contact with the ileum for one minute at point indicated by arrow. Other marks indicate addition of histamine to chamber. After each addition the gut was rinsed 3 times with fresh Tyrode's solution.

When testing the antihistaminic action of pyribenzamine on the terminal ileum of the guinea pig, the gut was suspended in a bath containing 3 ml. of Tyrode's solution. Uniform contractions were obtained by the addition of 0.016 μg. of histamine. Pyribenzamine was then added to the bath in amounts sufficient to make a final concentration of either 1:100,000,000 or 1:1,000,000,000 in the Tyrode's solution surrounding the gut. The solution of pyribenzamine was allowed to remain in contact with the ileum for one minute, after which the ileum was washed with fresh Tyrode's solution and its response to histamine promptly tested.

RESULTS

Increased quantities of histamine were found in all samples of blood plasma in which a hemolytic reaction occurred (Table I). Pyribenzamine did not affect the release of histamine during the hemolytic reaction (Table I).

TABLE I. FAILURE OF PYRIBENZAMINE TO AFFECT INCREASE IN CONTENT OF HISTAMINE IN PLASMA DURING IN VITRO HEMOLYTIC REACTIONS IN RABBIT BLOOD

RABBIT	TITER HEMOLYSIN	MICROGRAMS HISTAMINE PER ML. PLASMA MIXTURE OF RABBIT BLOOD IN WHICH OCCURRED: NO HEMOLYSIS	HEMOLYSIS IN PRESENCE OF PYRIBENZAMINE	HEMOLYSIS IN ABSENCE OF PYRIBENZAMINE
		0.15	0.48	0.63
		0.19	0.29	0.33
507	1:5, 120	0.17	0.39	0.39
514	1:2, 560	0.17	0.35	0.50
			0.33	0.31
			0.39	0.58
517	*	0.50	1.87	2.00
			1.60	
518	*		1.33	1.00
				1.00

*Titer not determined but marked gross hemolysis present after addition of sheep cells to whole blood.

A marked suppression of the contraction of the ileum of the guinea pig in response to histamine was produced by pyribenzamine in dilutions as small as 1:1,000,000,000. Repeated washings with Tyrode's solution were required to restore the gut to its former sensitivity to histamine (Fig. 1). Additions of extracts of blood to which pyribenzamine had been added showed no inhibitor effect on subsequent contractions of the gut to histamine. The removal of pyribenzamine by treatment with ether was therefore sufficient for the purposes of this study.

SUMMARY

1. In confirmation of earlier work, an increase in the content of histamine in plasma was found to accompany the in vitro hemolytic reaction which occurred in blood of rabbits sensitized to sheep erythrocytes when these were added to the blood.

2. The presence of pyribenzamine did not affect the in vitro liberation of histamine during the hemolytic reaction.

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IN VITRO RELEASE OF HISTAMINE BY HYPERSENSITIVE (ALLERGIC) SERUM IN CONTRAST TO IMMUNE (TREATED) ALLERGIC SERUM IN ANTIGEN AND NORMAL RABBIT BLOOD MIXTURES*†

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FUNDAMENTAL differences between the naturally occurring skin-sensitizing antibody (reagin) in nontreated allergic patients and the artificially produced blocking antibody in treated allergic patients were first demonstrated by Cooke, Barnard, Hebald, and Stull.[1] These were shown by skin reactions in normal individuals to the serum and antigen mixtures. Characteristics such as specificity, cellular affinity, and antigen affinity were shown. This evidence was not incontestable until Loveless[2] demonstrated the heat stability of the blocking antibody in contrast to the heat lability of the skin-sensitizing antibody. These findings were confirmed by other workers but a differentiation of these two antibodies on the basis of their clinical effects, namely, upon their quantitative release of histamine, has not been demonstrated. Abramson, Engel, and Moore[3] were unable to find histamine in the allergic wheal by means of iontophoresis. Katz[4] demonstrated that addition of antigen to the blood of a sensitized rabbit led to the release of histamine from the cells into the plasma. He also has shown that histamine is released when specific antigen is introduced into the cantharides blister fluid in a sensitive individual and this was not observed in treated sensitive cases.[5] Katz and Cohen[6] showed that the in vitro addition of specific pollen extract to the blood of a pollen-sensitive individual increased the histamine in the plasma. Dragstedt, De Arellano, Lawton, and Youmans[7] used normal rabbit blood as a source of bound histamine and upon addition of rabbit antisheep serum and sheep cells obtained an increase in the plasma histamine.

Since the allergic reaction is now generally considered to be due to histamine-like substance along with other metabolites, it was considered of interest to attempt to distinguish the untreated allergic serum from the treated immune serum, on the basis of histamine release. We were able to show that the posttreatment serum of allergic individuals, containing the immune antibody, united with antigen in the presence of normal rabbit blood without releasing histamine, in contrast to the untreated allergic serum which released histamine after addition of antigen in the presence of normal rabbit blood.

PROCEDURE

Blood was withdrawn from hay fever patients before and after complete preseasonal treatment of 3 to 4 months. The serum was stored without preservative at 5° C. To 2.0 ml. of serum was added 0.1 ml. of ragweed extract containing 100 protein nitrogen (P.N.) units. This mixture was incubated

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at 37° C. for 30 minutes. Rabbits were bled by intracardial puncture and 40 to 50 ml. of blood were withdrawn and heparinized. To the incubated serum-antigen mixture, 5.0 ml. of heparinized normal rabbit blood were added and after mixture were again incubated at 37° C. for 30 minutes. The contents of the tube were then centrifuged and the supernatant fluid was aspirated into a Hopkins' vaccine tube containing a capillary tip. The supernatant fluid was again centrifuged to pack any white cells mechanically aspirated in the supernatant into the capillary tip and eliminate their presence.

The supernatant fluid from the human serum, antigen, and rabbit blood mixture had a volume of 4.6 ml. Two ml. aliquots of this supernatant were then used for the McIntyre method[8] for purification of histamine. This method is now accepted as more accurate and less time consuming than former ones in general use. The final volume of each determination was made up to 5 ml. Histamine was then determined by biological assay using ileum strips from the guinea pig by the Schultz-Dale technique. An oxygenated bath of Tyrode's solution containing 0.025 Gm. $CaCl_2$ per liter was maintained at 37° C. Usually 0.5 to 1.0 ml. of the test solution was added to the ileum strip and standardized against freshly prepared histamine. All standardizations were checked, usually on a different ileum strip. From the standardization of the test solution by means of the Schultz-Dale technique, the amount of histamine in the 5.0 ml. of the final volume was obtained and multiplied by $\frac{4.6}{2}$ or 2.3 to obtain the histamine in the total supernatant of the serum, antigen, and normal rabbit blood mixture.

RESULTS

Table I shows the results obtained from 24 allergic sera on which both antetreatment and posttreatment sera were obtained from the same individual. It is evident that histamine is released from normal rabbit blood, acting as a reservoir of bound histamine, when incubated with untreated allergic serum and the specific antigen. In most cases the posttreatment serum, which is known to contain an immune antibody, caused no histamine release under identical experimental conditions. The allergic serum mixture containing only the skin-sensitizing antibody showed an average of 3.7 gamma of histamine in the total serum-plasma supernatant. The treated allergic serum mixture containing the immune antibody showed an average of 2.1 gamma of histamine in the total serum-plasma supernatant. In a series of 21 determinations on various samples of normal rabbit blood and serum mixtures without antigen, an average concentration of 2.2 gamma of histamine was obtained in the total serum-plasma supernatant. If this blank or control value is subtracted from the above results it will be seen that 1.4 gamma of histamine is released by 2.0 ml. of the skin-sensitizing antibody serum from 5.0 ml. of normal rabbit blood, in contrast to the immune or posttreatment serum which released no histamine under identical experimental conditions. The 100 unit (P.N.) strength of antigen used in the mixtures in Table I was chosen since it was the concentration found to give a positive response in all nontreated sensi-

tive sera from patients who gave a marked skin test to 10 or 100 units of antigen. When the histamine content of whole blood was determined it varied from 1.2 to 2.0 gamma per ml. This is in agreement with the values reported by Code.[9] It has been shown that 69 to 90 per cent of the whole blood histamine passes into the serum when blood is allowed to clot. Table I shows histamine values of 3.0 to 5.5 gamma in the supernatant as released from 5.0 ml. of normal blood. This probably represents the maximum concentration available to be released from the cells into the serum-plasma mixture.

TABLE I. HISTAMINE RELEASE FROM ANTETREATMENT AND POSTTREATMENT SERUM FROM THE SAME ALLERGIC INDIVIDUAL USING SPECIFIC ANTIGEN AND HEPARINIZED NORMAL RABBIT BLOOD

NAME	MARKED SKIN TEST TO		ANTIGEN ADDED	HISTAMINE* IN SUPERNATANT	
	RAGWEED	TIMOTHY		"A" SERUM	"P" SERUM
IMM.	100†	1000	100 ragweed	3.0	1.6
KAG.	10	100	100 ragweed	3.5	1.7
LIS.	100	100	100 ragweed	3.8	2.3
LOU.	10		100 ragweed	3.5	2.3
IEV.	10		100 ragweed	2.8	1.7
REI.	100		100 ragweed	4.1	2.0
HAL.	100	500	100 ragweed	3.2	1.7
SAN.	10	10	100 ragweed	3.5	1.7
McC.	10		100 ragweed	4.6	2.3
SIN.	100		100 ragweed	3.5	2.3
BON.	10	100	100 ragweed	3.7	0.9
FOX.	100		100 ragweed	3.7	1.7
BAR.	10		100 ragweed	3.5	0.6
ZOD.	100		100 ragweed	3.5	2.2
VOI.	10		100 ragweed	3.5	0.9
WEL.	10	10	100 ragweed	4.6	3.1
SCH.	100	100	100 ragweed	3.5	3.5
MKI.	100		100 ragweed	2.3	2.3
SPI.	1000	1000	100 ragweed	3.9	2.3
MAL.	10	100	100 ragweed	3.1	1.7
MAY.	100	1000	100 ragweed	5.5	3.6
SEB.	100	1000	100 ragweed	3.9	2.7
MTB.	10		100 ragweed	4.6	2.3
BRO.	100	100	100 timothy	4.3	2.8
Average in 24 cases				3.7	2.1
Average in 21 controls on normal rabbit blood				2.2	2.2
Actual histamine released by 2.0 ml. serum and antigen from 5.0 ml. normal rabbit blood				1.5	-0.1

*All histamine is expressed as base in gamma.
†All pollen extract potencies expressed in the protein nitrogen unit.

Table II shows that when larger amounts of antigen are added to nontreated allergic serum and rabbit blood the histamine is not increased over the amount released by the standard addition of 100 units for 2.0 ml. of serum.

Table III, in contrast to Table II, shows that when larger amounts of antigen are added to treated allergic serum, containing the immune and the skin-sensitizing antibodies, histamine is released probably due to the union of excess antigen with the skin-sensitizing antibody after the neutralization of the immune or blocking antibody.

Table IV illustrates the specificity of the histamine release from allergic sera. Sera from patients sensitive to pollen released histamine upon addition

of pollen to the mixture with normal rabbit blood, whereas, addition of other unrelated inhalants such as kapok or orrisroot caused no such reaction. One patient markedly sensitive to cottonseed and horse epithelium gave no response upon mixture of his serum with pollen extract. Many normal sera consistently gave negative results upon mixture with pollen extract.

TABLE II. ADDITION OF LARGER CONCENTRATIONS OF ANTIGEN TO NONTREATED ALLERGIC SERA

NAME	ANTIGEN ADDED	HISTAMINE* IN SUPERNATANT	HISTAMINE RELEASED IN SUPERNATANT
CAT.	none	2.1	
	100	5.0	2.9
	200	5.8	3.7
	500	5.1	3.0
	1000	5.0	2.9
	2000	5.2	3.1
HED.	none	1.8	
	100	3.5	1.7
	200	3.8	2.0
	500	3.8	2.0
	1000	3.8	2.0

*All histamine is expressed in gamma as base in total supernatant from 2.0 ml. serum, 0.1 ml. antigen, and 5.0 ml. normal rabbit blood.

TABLE III. ADDITION OF LARGER CONCENTRATION OF ANTIGEN TO TREATED ALLERGIC SERA

NAME	ANTIGEN ADDED	HISTAMINE IN SUPERNATANT*	
		"A" SERUM	"P" SERUM
BRO.	100 timothy	4.3	2.8
	500 timothy	–	4.6
WEL.	100 ragweed	4.6	3.1
	200 ragweed	–	3.5
	500 ragweed	–	4.2
MAR.	100 timothy	–	2.4
	1000 timothy	–	3.5
REI.	100 ragweed	–	2.3
	1000 ragweed	–	3.5

*All histamine is expressed in gamma as base.

TABLE IV. SPECIFICITY OF HISTAMINE RELEASE

NAME	CLINICAL SENSITIVITY	ANTIGEN ADDED		HISTAMINE IN SUPERNATANT*
SIL.	ragweed positive	ragweed	100	3.5
	kapok negative	kapok	500	2.0
THO.	ragweed negative	ragweed	150	1.7
ZOD.	ragweed positive	ragweed	100	3.5
	orrisroot negative	orrisroot	500	2.3
93	ragweed negative	ragweed	100	1.3
NEU.	normal	ragweed	100	1.8
COA.	ragweed negative	ragweed	100	2.6
KRO.	cotton and horse epithelium positive	none		2.0
	ragweed negative	ragweed	100	2.3

*All histamine is expressed in gamma as base.

Table V shows the results obtained when antetreatment sera are heated at 56° C. for 4 hours and then mixed with antigen and normal rabbit blood.

There were 15 sera in this series of which 8 showed an inhibition of the histamine release due to heating at 56° C. for 4 hours, whereas, 7 were unaffected and released histamine to an extent comparable to that in the unheated sample. Therefore, the heating of nontreated allergic sera at 56° C. for 4 hours' duration was insufficient to inactivate all sera.

TABLE V. EFFECT OF HEATING ANTETREATMENT SERA FOR 4 HOURS AT 56° C. UPON RELEASE OF HISTAMINE FROM IN VITRO MIXTURES

HUMAN SERA	HISTAMINE IN SUPERNATANT (GAMMA)	
	UNHEATED SERUM AND ANTIGEN	HEATED SERUM AND ANTIGEN
BON.	4.6	4.4*
WIL.	3.3	3.6*
McK.	6.1	4.2
SAN.	4.5	4.5*
IMM.	3.5	3.5*
LIS.	3.5	1.6
130	3.4	2.3
124	4.5	2.5
139	3.5	2.3
BAT.	5.0	3.5
FRE.	4.6	4.5*
130	5.2	3.5
NIL.	5.2	2.9
HED.	5.2	4.8*
GOU.	4.0	4.0

All mixtures contain normal rabbit blood as a source of bound histamine.
*Represents 7 sera unaffected by heating for 4 hours.

Table VI shows that antetreatment serum which is heated at 56° C. for 10 hours has completely lost its ability to release histamine after mixture with antigen and normal rabbit blood. In a series of 9 cases, 8 showed this inhibition. The one exception was obtained from a patient exhibiting multiple sensitization. The reliability of his skin reactions, therefore, could be questioned. Heating at 56° C. for 10 hours seems to be a critical temperature-time limit for all nontreated allergic sera.

TABLE VI. EFFECT OF HEATING ANTETREATMENT SERA FOR 10 HOURS AT 56° C. UPON RELEASE OF HISTAMINE FROM IN VITRO MIXTURES

HUMAN SERA	HISTAMINE IN SUPERNATANT (GAMMA)	
	UNHEATED SERUM AND ANTIGEN	HEATED SERUM AND ANTIGEN
NEA.	4.0	2.4
153	4.1	1.7
136	2.5	0.9
149	3.2	1.8
151	2.9	1.5
147	3.5	2.3
DEA.	3.4	3.5
JON.	3.5	1.7
GRE.	4.1	2.8
Average	3.5	2.1
Control	2.1	2.1
Histamine released	1.4	0.0

All mixtures contain normal rabbit blood as a source of bound histamine.

Table VII shows that when posttreatment serum is heated at 56° C. for 10 hours and then mixed with an antetreatment serum, antigen, and normal rab-

bit blood, the ability of the posttreatment serum to inhibit the release of histamine remains and is not affected by this temperature and time period.

TABLE VII. EFFECT OF HEATING POSTTREATMENT SERA FOR 10 HOURS AT 56° C. UPON RELEASE OF HISTAMINE FROM IN VITRO MIXTURES

	HISTAMINE IN SUPERNATANT (GAMMA)		
HUMAN SERA	"A" SERUM AND ANTIGEN*	"A" SERUM AND "P" SERUM, UNHEATED, AND ANTIGEN	"A" SERUM AND "P" SERUM, HEATED, AND ANTIGEN
140A	4.1		
TOR-P		3.3	3.3
164A	3.5		
23P		2.6	2.6

All mixtures contain normal rabbit blood as a source of bound histamine.
*Antigen is 100 P.N. units ragweed extract.

In all of the preceding data the serum and antigen mixtures were incubated at 37° C. for 30 minutes and then mixed with normal rabbit blood and incubated again for 30 minutes. Three different antetreatment sera and antigen mixtures after incubation at 37° C. for 30 minutes were mixed with normal rabbit blood and incubated for 3, 4, and 5 minutes, respectively. Histamine was released in the same quantity as was observed after 30-minute incubation.

DISCUSSION

Dragstedt, De Arellano, Lawton, and Youmans[7] experiments showed that normal rabbit blood could be utilized for the release of histamine following the addition of rabbit antisera and antigen. Our experiments with human sera show that normal rabbit blood may be used as a reservoir of bound histamine when studying allergic reactions of a species other than the rabbit.

By utilizing normal rabbit blood as a reservoir of bound histamine, it has been possible to show that the skin-sensitizing antibody present in antetreatment allergic serum when mixed with antigen, in vitro, releases histamine. When the immune antibody present in posttreatment allergic serum is added to the same quantity of antigen and rabbit blood no histamine is released. Since the immune antibody unites or reacts with antigen without release of histamine, this is of clinical significance since most of the allergic manifestations are now recognized as resulting from the release of histamine and other minor metabolites.

Coca and Grove,[10] and Schmidt and Lippard[11] showed that the skin-sensitizing antibody was heat labile. Loveless[2] stated that the reagin is destroyed by heating at 56° C. for 5 hours in all cases. Chase[12] pointed out that such antibodies lost their tissue-sensitizing function when heated at 56° C. for a period of between one-half and 8 hours; that there was no critical temperature, the degree of heating required for full inactivation varying from one serum to another. Our results show that in only about 50 per cent of the instances were the skin-sensitizing antibodies destroyed by heating at 56° C. for 4 hours. Practically 100 per cent were inactivated by heating at 56° C. for 10 hours. The immune antibody present in the posttreatment serum heated for 10 hours at 56° C. was still able to inhibit the histamine release when added to

skin-sensitizing antibody and antigen. It would seem to be heat stable at this temperature and time interval which inactivates the skin-sensitizing antibody.

It seemed of interest to determine whether from normal nonsensitized cells histamine would be released into the plasma by antigen and antibody reaction. It was found that histamine was released by skin-sensitizing antibody and antigen mixtures, incubated with normal whole blood for only 3 minutes. However, these data do not preclude the possibility that the leucocytes may have been fixed by antibody even in this short interval.

The immune antibody unites with certain quantities of antigen since the histamine release is inhibited in posttreatment serum in which it is present, along with the skin-sensitizing antibody. The immune antibody has greater avidity for antigen than the skin-sensitizing antibody. It is only when antigen is added in excess that it finally becomes available for reaction with the skin-sensitizing antibody as shown by the release of histamine from cells to plasma in these mixtures. Since the immune antibody unites with or adsorbs antigen in some way without releasing histamine, its presence can never be accepted nor denied when judged solely by a reaction in which histamine release is a characteristic factor. For example, it is often stated that the immune antibody does not become affixed to the cells and is not sensitizing. When the presence of cell sensitization in human beings can be demonstrated, it is usually by determining the occurrence of a wheal reaction mediated through the release of histamine. This manner of test is not valid for the immune antibody since it does not release histamine.

These experiments show for the first time that nontreated allergic sera when added to specific antigen releases histamine from a bound source whereas treated allergic sera upon addition of specific antigen releases no histamine from a bound source. These findings demonstrated the beneficial nature of the immune antibody since it combines with antigen without histamine release and, therefore, without the clinical symptoms of allergy. It is not until the antibody is saturated or neutralized that excess antigen is free to react with the ever present skin-sensitizing antibody to give histamine release with its resultant clinical picture.

Since the completion of this study Hjort, Worden, and Hogle,[13] using a somewhat different technique, could not verify the work of Katz and Cohen.[6]

SUMMARY

1. Our experiments with human sera show that rabbit whole blood can be used satisfactorily as a reservoir of bound histamine.

2. Histamine is released in vitro from normal rabbit blood cells into the plasma by nontreated allergic serum and specific antigen; it is not released by treated allergic serum and the same concentration of antigen.

3. On addition of larger quantities of antigen to treated allergic serum the excess antigen reacts with skin-sensitizing antibody and histamine is then released.

4. Only one-half of the nontreated allergic sera were inactivated when heated at 56° C. for 4 hours.

5. Practically all of the nontreated allergic sera were inactivated when heated at 56° C. for 10 hours.

6. Treated allergic sera were not inactivated by heating at 56° C. for 10 hours.

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POLLEN TOLERANCE NASAL TESTS IN HAY FEVER. EXPERIMENTAL AND CLINICAL OBSERVATIONS*†

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INTRODUCTION

ACCURATE analysis of the degree of subjective relief afforded by specific pollen therapy has always presented a problem to the practicing allergist. Up to now the information depended either upon the judgment of the symptomatic relief reported by the patient or by personal observations made during the pollinating period. Both methods represent crude, uncertain measurements which will not withstand close scientific scrutiny. Personal unpublished observations have shown that there is considerable variation between the amount of relief reported by the patient at the conclusion of the season and that recorded by means of a daily log. Individuals often claimed good to excellent results from their treatment, while their daily log recorded an appreciable degree of discomfort. Personal observations made by the attending physician during the season also were equivocal, since they depended on one's judgment during short periods that were not representative of the entire season.

Correlation between clinical symptoms and the absolute amounts of atmospheric pollen frequently has been lacking. This has created much confusion and skepticism regarding the significance of the daily pollen counts as recorded in many metropolitan newspapers along the Eastern seaboard. This discrepancy has been attributed variously to low barometric pressures, to dampness, to sudden changes in temperature, to changes of season, etc. Actually we know very little about its exact mechanism. It is possible that the presence of other nonpollen factors might accentuate the oculonasal symptoms of an already-established hay fever although the same factors in themselves might be incapable of producing threshold symptoms.

Regardless of the nature of these factors and the manner by which they affected individual patients, it seemed desirable to replace our crude yardstick for measuring the degree of clinical relief with a more accurate and objective method. Since pollen was the chief excitant in hay fever, and a constant factor, it occurred to us that by bringing it into direct contact with the principal shock organ, it should be possible to determine more definitely the results of specific desensitization. For this purpose the direct nasal or inhalation test employing pollen in varying concentrations might offer the best approach. Accordingly, in 1944, such an investigation was begun and the results obtained form the basis of the present report.

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PROCEDURE

The nasal or inhalation test used was similar to that previously reported,[1] except that one had to be more certain that the amount of inhaled pollen was as nearly constant as possible. This was accomplished by blowing the pollen from the blunt end of a toothpick on to a petrolatum-covered slide and then counting all the granules thus deposited. The applications were made by one individual while the counting was done by several technicians. The percentage deviation was then determined. The next problem was to find a diluent that permitted good admixture and distribution of pollen, that was nonirritating to normal nasal mucosa on multiple instillations, and that was sufficiently light and fluffy to permit easy inhalation. The following mixtures of pollen and diluent were first prepared.

1:1000	1:75	1:8
1:500	1:50	1:6
1:250	1:40	1:4
1:200	1:30	1:2
1:150	1:20	Concentrated
1:100	1:10	

The pollen mixture was then delivered to the nasal mucosa by means of a powder blower. This provided for delivery of a measured amount of pollen under what was considered standard conditions. Early experiences uncovered two drawbacks to this method; the first being the irritating nature of the forced draft used to deliver the pollen mixture, while the second revolved around the loss of pollen mixture due to its adherence to the rough surface of the nozzle of the blower. When this was found wanting, it was deemed advisable to permit the patient to insufflate the mixture by forced inspiration through one nostril. Although the depth and force of inspiration varied with each patient, it had many advantages over the blower method of application. The patient's tolerance to pollen was determined by single applications of the mixture beginning with the 1:1000 dilution and increasing the strength of the mixture upon each subsequent visit until a positive reaction occurred. Rhinorrhea and blockage were not sufficiently definite and objective criteria to be used to determine a positive reaction. Sneezing, together with rhinorrhea and blockage, was considered a positive test. Because of length of time required after instillation to determine the pollen tolerance in a single patient, a modification in procedure was made. This consisted of successive applications of pollen mixture on the same visit until a positive reaction was obtained. The number of dilutions originally used was too cumbersome and was correspondingly reduced to the following:

1:1000	1:10
1:250	1:2
1:50	Concentrated

Each patient was tested first with the diluent. If no reaction followed, the 1:1000 dilution was instilled. Five minutes were permitted to elapse before a second or subsequent application was made. If no reaction occurred, the next higher concentration was instilled. This was continued until a positive reac-

tion resulted. In order to insure adequate distribution of pollen in the diluent it was found necessary to agitate the mixture each day before using it.

The results of testing cases of hay fever treated by preseasonal and perennial methods are presented and the clinical implications and usefulness of this method of testing are discussed as follows: (a) the variability of reaction of the nasal mucosa to absolute amounts of pollen; the nature of some of these factors and the role they play in clinical hay fever; (b) the results of pollen tolerance tests before and after treatment by the preseasonal method; attempts to correlate the subjective improvement with objective observations and with the total and maximum dosages of pollen extracts; (c) the changing state of pollen tolerance before and after numerous years of consecutive perennial treatment; and (d) the usefulness of this method in determining the existence of spontaneous remissions or clinical cures.

RESULTS

The amount of pollen that was contained on the blunt end of a toothpick was fairly constant for pollen of the same size. Ragweed counts were 18,445, 18,746, and 18,248, a deviation of only 2.6 per cent.

Timothy counts were 9,882 and 10,176, a deviation of 3.5 per cent.

Many different types of diluents were considered before a selection was made. Pollen not indigenous to the locality seemed to offer many advantages over the artificial media, such as good admixture and distribution because of size and physical qualities. In our early trials, however, talc seemed to compare favorably in all respects with all other types of diluents. It was found that after long use the talc showed a slight tendency to cake. When this occurred, the mixtures were renewed.

The reactivity of the nasal mucosa to measured amounts of pollen was fairly constant. This was demonstrated by repeatedly precipitating clinical symptoms with a given dilution of the pollen-talc mixture. Most reactions were mild and required no therapy to counteract them. The occasional severe reaction could be controlled adequately by the topical application of vasoconstrictors and the oral administration of antihistaminic agents.

The threshold of nasal sensitivity to absolute amounts of pollen varied with individual patients. Thus, some reacted to dilutions of 1:1000 and others required a 1:2 dilution to precipitate a reaction. There was no absolute correlation between their initial theshold reaction and the severity of clinical hay fever, although more individuals with relatively low tolerances had more discomfort during the season. Fig. 1 displays this variability as determined with single applications of pollen before any specific treatment had been rendered. Fig. 2 depicts this same variability as determined by the multiple and successive method in patients receiving the preseasonal form of therapy. The tests were performed at least 5 months after the completion of specific therapy.

A total of 44 patients manifesting clinical hay fever were tested by this method before preseasonal treatment had been started and again after the termination of the pollinating season; 25 showed increased tolerance, 14 showed no change in tolerance, and 5 showed decreased tolerance.

Of the 25 showing increased tolerance, 19 reported good results from specific therapy, 6 reported fair results from specific therapy, and there were no poor results.

Of the 14 showing no change in tolerance, 9 reported good results, 2 reported fair results, and 3 reported poor results.

Of the 5 showing decreased tolerance all reported good results.

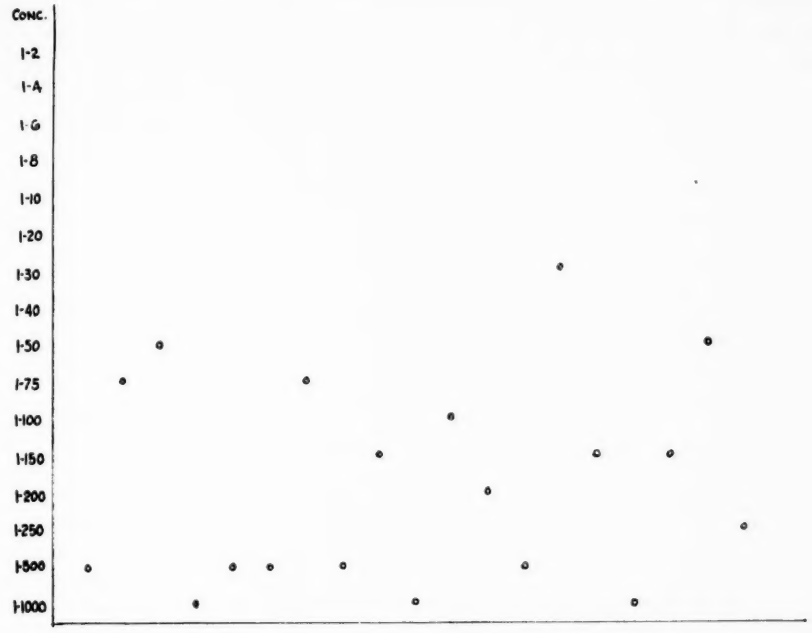

Fig. 1.—Nasal threshold reactions as demonstrated by pollen tolerance tests with single applications.

It had been hoped originally that this test would provide the medical attendant with an objective method by which he could gauge more accurately the degree of symptomatic relief obtained by the patient at the completion of specific pollen therapy. The lack of correlation between the change in nasal pollen tolerance before and after the season, and the degree of symptomatic relief reported by the patient renders this an uncertain means of estimating the actual response to specific pollen therapy. Furthermore, the changes in pollen tolerance noted above could not be correlated either with the total amount of pollen extract received by the patient, or with high dosage. Thus, some reported excellent results and objectively showed increased pollen tolerance from a low total and maximal pollen dose, whereas others reported poor results from higher total and maximal doses.

Although the pollen tolerance before and after a single year of preseasonal treatment varied considerably, it increased steadily with each successive year of perennial treatment. This observation corroborates the clinical impression held by many regarding the excellence of perennial therapy in the third and successive years of specific pollen treatment. Twenty-one patients

were tested in this manner over periods varying from 3 to 6 years with an average of 3.85 years. There was a significant increase in pollen tolerance in all the subjects tested, although the same variability existed here in the first year of perennial treatment as it had with our preseasonal subjects. Representative graphs of this increased tolerance are shown in Figs. 3 and 4. There was better correlation in this group between their symptomatic relief and the degree of pollen tolerance than in the preseasonal one. No parallelism existed between the total and maximum doses of pollen and either their subjective symptoms or the objective nasal pollen tolerance.

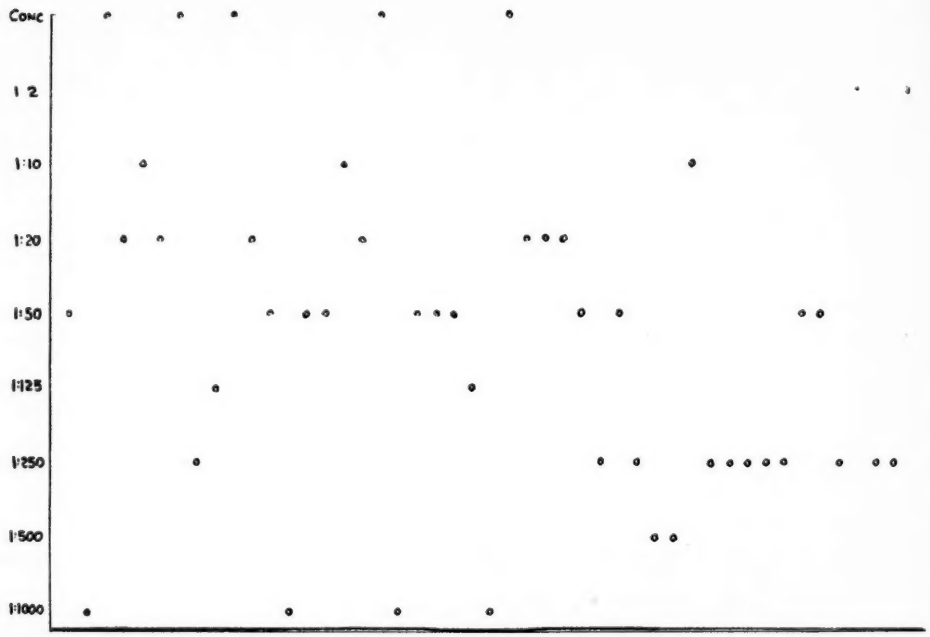

Fig. 2.—Nasal threshold reactions as demonstrated by pollen tolerance tests with successive instillations.

The diagnosis of spontaneous remissions or so-called clinical cures calling for a cessation of treatment has always been a difficult and a highly individualized one. There are those who call for a trial without treatment when the patient has manifested a minimum of symptoms for 3 successive years. Others are loath to stop treatment until every last vestige of hay fever has been eradicated. A small minority favor interrupting treatment when the cure becomes worse than the disease. Many patients take matters in their own hands and stop treatment against their physicians' advice when they think they have attained the desired results. None of the above methods are infallible, nor can any combination give the physician and the patient the assurance they desire against recurrent symptoms. Since each instillation of concentrated, dry pollen corresponded with the average amount of pollen inhaled during a 24-hour period, it seemed likely that any patient capable of withstanding repeated instillations within a relatively short time interval

might not need further treatment. Several modifications were made in the number of instillations and the time interval between applications before the standard procedure of 3 inhalations of the concentrated pollen with a 5-minute interval between each was adopted. Ten patients yielding a negative reaction to this procedure were advised to stop treatment. All 10 have been followed for 2 years without manifesting recurrent hay fever. Before this procedure was established we encountered several patients who had recurrent symptoms within 1 to 3 years after cessation of treatment. When retested by the above method a positive reaction was obtained in each case. The superiority of this method over the less certain and more diverse criteria for clinical cures establishes it as a useful adjunct in the practice of allergy. It should be used in those patients whose response to treatment has been so good as to suspect the existence of a spontaneous remission or clinical cure.

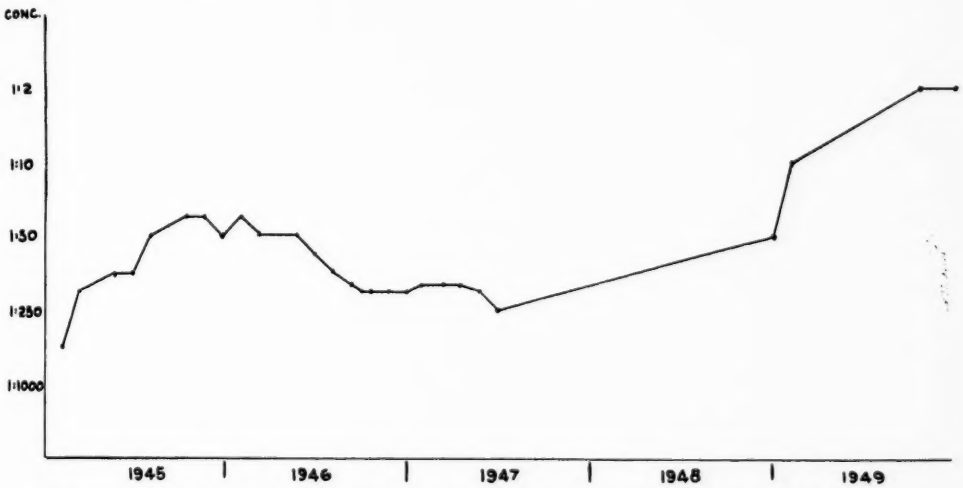

Fig. 3.—Pollen tolerance tests in a patient under continuous perennial pollen therapy.

DISCUSSION

It was evident from these studies that the tolerance of the nasal mucosa is influenced by factors other than the amount of pollen instilled. This alteration was not manifested by all patients but only by those in whom this additional factor was present at the time of testing. Repeated testing on such subjects showed a variable reaction from week to week to fixed amounts of pollen. At such times some of the patients gave positive reactions to our control, talc, whereas under normal conditions this reaction was lacking entirely. Most patients, however, maintained a rather constant reaction to the inhalation of a fixed amount of pollen. A careful review and analysis of these histories showed that those with variable reactions also manifested hay fever-like symptoms out of season, while those with strictly seasonal symptoms were more consistent in their response to pollen. The agents bringing about these alterations included infectious, meteorological, and psychic factors. This is in keeping with the recent experimental work of Wolff, Holmes, Goodell, and

Wolf[2] which demonstrated the ability of psychic stimuli to enhance the mucosal edema produced by specific pollen. It also corroborates a common clinical impression that the severity of hay fever symptoms is not always directly proportional to the atmospheric pollen concentration. Seasonal hay fever patients, therefore, may be divided into those whose symptoms are strictly seasonal and others who also manifest perennial symptoms. In this latter group one will find a variable reaction to pollen depending upon the presence of other specific sensitivities or nonspecific factors that enhance the allergic response of their shock tissues. The pollen tolerance of patients with strictly seasonal hay fever is much more constant.

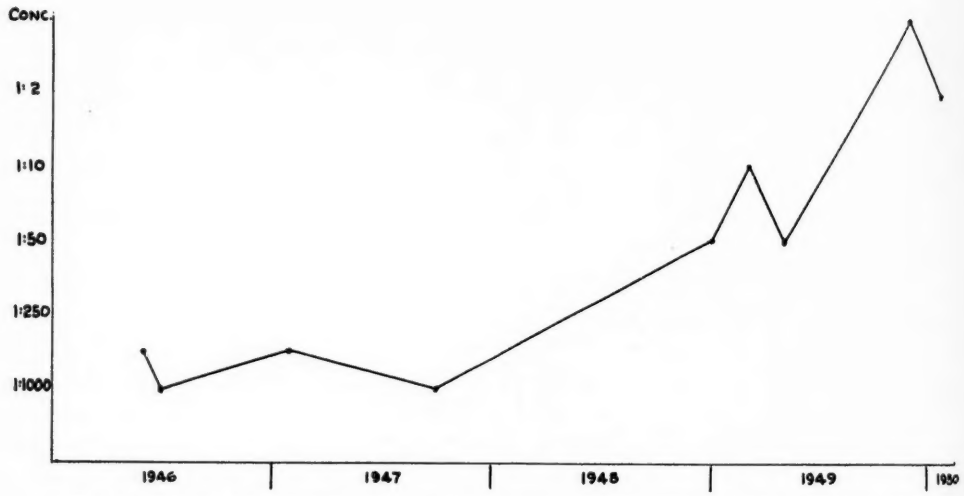

Fig. 4.—Pollen tolerance tests in a patient under continuous perennial pollen therapy.

The pollen tolerance test did not fulfill all the expectations originally held for it. It had been hoped that this method would detect a marked increase in pollen tolerance in those responding well to specific therapy, whereas there would be a negligible change in those with fair to poor therapeutic results. For some unexplainable reason there was no parallelism between the change in pollen tolerance and the degree of clinical relief in the preseasonally treated individiuals. By contrast, there was a characteristic increase in tolerance among those treated for many years by the perennial method. It is difficult for one to comprehend why a satisfactory therapeutic response should not be accompanied by increased tolerance to the specific pollen. Yet our observations on a fair proportion of the preseasonally treated group failed to show such correlation. The unexpected results obtained in the latter patients can only be clarified by continued observations on larger groups and perhaps by certain modifications in procedure that would eliminate as many of the variables as possible.

From the results of our studies, it would seem that the pollen tolerance test can be quite useful as a criterion of clinical remission or cure. Since it is an objective method, it obviates the necessity of depending upon historical

data; however, it still may be used in conjunction with the latter in deciding upon the necessity for continued treatment. The mild degree of discomfort occasioned by a positive reaction is easily controlled by the topical application of vasoconstrictors and cannot compare to the prolonged discomfort of recurrent symptoms from an improperly diagnosed spontaneous remission.

SUMMARY

1. The pollen tolerance nasal test is a fairly accurate means of measuring the reaction of the principal shock organ to a given amount of pollen under standard conditions.

2. The reactivity of the shock organ is influenced by other factors, some specific, some nonspecific. Among the specifications are concomitant sensitivities to other foods, pollens, and inhalants. The nonspecific ones include respiratory infections, meteorological factors, and psychic stimuli. These complicating factors are best detected by an accurate and detailed history together with a complete allergic survey.

3. There was a constant and significant increase in pollen tolerance only with prolonged or perennial treatment. The results of pollen tolerance tests with preseasonal treatment for a single year were much more variable. It was impossible to forecast the therapeutic efficacy of specific treatment by this method since there was no parallelism either between the changing tolerance of the patients to pollen and their clinical results, or between the pollen tolerance and the total and maximum pollen dosage.

4. This test offers a satisfactory objective method for the determination of a spontaneous remission or cure. It may be used together with historical data in deciding when specific pollen therapy should be discontinued.

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THE INCIDENCE OF ABNORMAL ELECTROENCEPHALOGRAPHIC PATTERNS IN ALLERGIC CHILDREN*†‡

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DEES and Lowenbach[4] reported the occurrence of abnormal encephalographic tracings in 50 per cent of the allergic children they examined. These findings and their possible significance stimulated our interest. We thought it of value: (1) to check the incidence of such abnormalities; (2) to test the influence upon them of an antihistaminic drug; and (3) to study the patterns of parents and siblings of such children.

Eighty children from the Pediatric Allergy Clinic of the University Hospital, New York University-Bellevue Medical Center, were selected for study. The children's ages ranged from 2 to 14 years; they were heterogeneous as to sex, diagnosis, severity of illness; they had been treated for varying lengths of time (with a few who had not been treated at all); none of them had a history of convulsions or syncope, either in themselves or in their parents or siblings; only two had an indefinite history as behavior problems.

A complete allergic investigation was made of each child, a history and a complete physical examination (including an otolaryngological survey, roentgenograms of chest and sinuses, blood counts, and nasal smears for eosinophilia).

A 6 channel Grass apparatus was used. Electroencephalographic recordings were made from the frontal, temporoparietal, and occipital regions bilaterally, employing a minimum of 10 electrodes and utilizing both monopolar and bipolar systems of recording.

Of the 80 encephalograms recorded, 54 were completely normal, 26 abnormal (Table I).

TABLE I. NUMBER OF ORIGINAL TRACINGS

		MALE	FEMALE
Abnormal	26 cases	13 (50%)	13 (50%)
Normal	54 cases	35 (65%)	19 (35%)
Total	80 cases		
Abnormals	32.5%		

A comparison of the 2 groups reveals some marked similarities. A great majority of children in both had the onset of allergic manifestations before the age of 6. Most of both groups suffered from clinical allergy for a period of 2 to 7 years prior to the recordings. Their clinical diagnoses were predominately asthma, eczema, or allergic rhinitis. Their ages at the time of testing were distributed rather equally between 5 and 14 years (Tables II and III).

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TABLE II. NORMAL ENCEPHALOGRAMS

	NUMBER OF CASES		
YEARS	AGE AT ONSET OF SYMPTOMS	NO. YEARS OF ALLERGY	PRESENT AGE
Less than 1	9		
1	6	2	
2	5	10	
3	3	9	
4	10	7	2
5	8	6	4
6	6	4	6
7	3	4	4
8	1	3	7
9	2	1	8
10		3	3
11	1	2	8
12		1	4
13			3
14		2	5
Total	54	54	54

The major difference between the groups appears, however, in sex distribution. In the normal group there were 36 boys and 18 girls (ratio 2:1); in the abnormal group, 13 boys and 13 girls (ratio 1:1) (Tables II and III).

TABLE III. ABNORMAL ENCEPHALOGRAMS

	NUMBER OF CASES		
YEARS	AGE AT ONSET OF SYMPTOMS	NO. YEARS OF ALLERGY	PRESENT AGE
Less than 1	5		
2	2	3	
3	5	3	
4	3	7	
5	3	2	
6	5	1	4
7	1	4	3
8	1	0	3
9	1	1	1
10		3	4
11		1	1
12		1	4
13			5
14			1
Total	26	26	26

The children of the abnormal group were then given under supervision Trimeton for 3 weeks (25 mg. 3 times a day); the encephalographic tracings were then repeated with an interval varying between 4 weeks and 8 months between tests. Of the 21 repeat electroencephalograms we were able to secure, 20 were identical with the first. In only one case did the pattern revert, following medication, to normal (Table IV).

TABLE IV. REPEAT ENCEPHALOGRAMS

Repeat encephalograms (following Trimeton)	21
No change	20
Reversal to normal	1

An attempt was then made to study the parents and siblings of children in the abnormal group. As many patterns as possible were secured. We were able to obtain 42 encephalograms in 22 families (Table V).

TABLE V. FAMILY PATTERNS (42 ELECTROENCEPHALOGRAMS)

TOTAL MALE		TOTAL FEMALE		TOTAL
Normal	18	Normal	8	26
Abnormal	5 (22%)	Abnormal	11 (58%)	16 (38%)

Here, too, there is a marked predominance of abnormal patterns among the females. There were 23 males with 5 abnormal patterns (22 per cent), as compared with 19 females with 11 abnormal patterns (58 per cent). There were 12 fathers with 2 abnormal patterns (17 per cent), as compared with 17 mothers with 10 abnormal patterns (60 per cent). The 6 brothers (with 3 abnormal patterns) and the 2 sisters (with 1 abnormal pattern) may be discounted as statistically invalid. On the other hand, in tests of several parents of the normal children, there was not a single abnormal pattern. In the overall picture, there were 16 abnormal patterns (38 per cent) in the 42 tracings taken in this group.

TABLE VI. INCIDENCE OF ABNORMAL PATTERNS IN RELATION TO YEARS OF CLINICAL ALLERGY

YEARS OF ALLERGY	NORMAL EEG	ABNORMAL EEG	PER CENT ABNORMAL
0 to 2	12	3	20
3 to 4	16	10	38
5 to 6	10	3	23
7 to 8	7	4	36
9 to 10	4	4	50
11 to 12	3	2	40
13 to 14	2	0	0

In an attempt to get greater statistical validity and to see whether 2-year grouping would yield different conclusions, we reclassified our cases, dividing the children into groups with 2, 4, 6, 8, 10, 12, and 14 years of clinical allergy. Table VI suggests that the number of abnormal patterns increases with the increased duration of allergic symptoms; this seems to confirm the impression of Dees and Lowenbach.[4]

TABLE VII. INCIDENCE OF ABNORMAL PATTERNS IN RELATION TO CHRONOLOGICAL AGE

	AGE GROUPINGS		
PRESENT AGE (YRS.)	PATTERNS		PER CENT ABNORMAL
	NORMAL	ABNORMAL	
4 to 5	6	0	0
6 to 7	10	7	41
8 to 9	15	4	21
10 to 11	11	5	31
12 to 14	12	10	45

Our children were again reclassified, according to the age at which the tests were done irrespective of the duration of their allergic symptoms (Table VII). Although the percentage of abnormal patterns seems to increase with

increasing age, there are exceptions, as among the 6 and 7 year olds, among whom the percentage of abnormal patterns increases disproportionately. But with age 9 as the arbitrary base line, there were 10 out of 33 abnormal under it (30 per cent), and 16 of 47 abnormal over it (35 per cent); increasing age, therefore, seems to be of no particular significance (Table VIII). Similarly, the mean age of the normal group is 9.2 years; the abnormal group 9.8 years; the difference is without statistical significance.

TABLE VIII. AGE GROUPINGS ABOVE AND BELOW 9 YEARS OF AGE

AGE	BELOW AGE 9		ABOVE 9 (%)
	NORMAL PATTERNS	ABNORMAL PATTERNS	
Below 9	23	10	30
Above 9	31	16	33

To determine the relationship between abnormal pattern and type of allergy, we reclassified our cases according to their allergic manifestations (Table IX). Among the normals: asthma, 67 per cent; eczema, 22 per cent; and nasal allergy, 11 per cent. Among the abnormals: asthma, 62 per cent; eczema, 23 per cent; and nasal allergy, 15 per cent. Again, no correlation can be found.

TABLE IX. TYPE OF ALLERGY IN RELATION TO ELECTROENCEPHALOGRAMS

NORMAL EEG			ABNORMAL EEG		
	NO. OF CASES	(%)		NO. OF CASES	(%)
Asthma	36	67	Asthma	16	63
Eczema	11	20	Eczema	6	23
Nasal allergy	6	11	Nasal allergy	4	15

DISCUSSION

The interpretation of the electroencephalograms of children is difficult since norms have not been well established. Slow waves, abnormal in the adult record, are normally encountered in children. The incidence and frequency of these slow potentials vary considerably with the age of the child or degree of physiologic maturation, the older the child, the smaller the incidence of slow activity. There are relatively wide variations in the incidence of slow waves for each age group and the extremes of these variations at each age level have never been well delimited. In general, however, it may be stated that the older the child the less uncertain the interpretation of the record, since the tracing tends to approach the adult type.

The interpretation of our electroencephalograms was dependent largely on the experience of the individual observer (B.L.P.) and the criteria established by Lindsley,[1] Gibbs and Gibbs,[2] and Henry.[3]

Abnormal activity consisted chiefly in the appearance of slow potentials, at times in serial bursts, in greater incidence than is usually encountered in clinically normal children. Tracings were not diagnosed as abnormal unless there was a definite deviation from what are considered "normal" tracings. Dees and Lowenbach[4] have stressed the incidence of occipital dysrhythmia in

their paper. Their observations in this connection are difficult to evaluate since they make no reference to clinical controls which stress differences in tracings between the frontal and occipital regions in children. We did not believe that stress could be placed upon so-called occipital dysrhythmia alone, since it is very commonly observed in clinically normal children, when the remainder of the electroencephalographic pattern over the frontal and parietal areas is well within normal limits.

The incidence of abnormal encephalograms in our group (33 per cent) is smaller than that reported by Dees and Lowenbach (50 per cent). Two facts may explain the difference: (1) their inclusion of cases with definite histories of convulsions and syncope tended to increase their figure, and (2) our de-emphasis of occipital dysrhythmia tended to decrease ours.

Our findings that 38 per cent of the blood relatives of our group had abnormal patterns seems to be of considerable interest. It tends to substantiate previous observations that just as allergy is hereditary, so encephalographic patterns may be hereditary.

Further significance of abnormal encephalograms is not clear, since similar abnormalities in a similar degree have been reported in behavior disorders of children, in migrainous states and convulsive, disorders.

We were unable, in any case except one, to alter the abnormal patterns by use of Trimeton. It is likely that the occurrance of an abnormal pattern without any clinical allergic manifestation should be regarded in the same light as a positive skin reaction without clinical correlation.

SUMMARY

1. There was a high incidence (33 per cent) of abnormal electroencephalographic patterns in allergic children.

2. A similarly high incidence of abnormal patterns in the families of allergic children was found.

3. A definite preponderance of abnormalities appeared in the female over the male.

4. The apparently increasing incidence of abnormal patterns with the increasing duration of allergic symptoms was evidenced.

5. No apparent correlation between the clinical types of allergy and encephalographic patterns was found.

6. An antihistaminic drug (Trimeton) failed to alter the electroencephalographic pattern.

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BRONCHIECTASIS IN ASTHMA

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IT HAS been the prevailing opinion that bronchiectasis frequently originates from allergic asthma. Watson and Kibler[1] estimated the percentage of allergic asthma among their cases of bronchiectasis to be as high as 90 per cent. Thomas, Ostrand, and Tomlinson[2] noted that in 190 consecutive cases of bronchiectasis approximately one-half manifested evidence of a major allergic disease. It is not clear whether or not the diagnosis bronchiectasis in these cases was confirmed by bronchograms. In contrast to this observation, in 60 autopsies of chronic asthma reported by Mallory[3] bronchiectasis was so exceptional that its occurrence was considered coincidental. Emphysema and cor pulmonale were noted much more frequently. In the practice of one of us (G. L. W.) only very few cases of irreversible bronchiectasis were encountered among several thousand asthmatic patients.

This discrepancy of opinion raises the question of whether or not bronchiectasis in allergic asthma is of an evanescent type and whether there might be different types of bronchiectasis which should be viewed clinically and prognostically in a different light. In order to elucidate this question further we studied the bronchograms of 28 patients taken during the past 3 years out of a total of 756 individuals with chronic asthma.

It should be pointed out that taking a bronchogram in an allergic patient is fraught with considerable risk. There is a possibility of (1) crowding the oil into an already overcrowded bronchial tree and thus inducing asphyxiation; (2) of sensitivity to the contrast medium, and (3) of pneumonitis through nonabsorption of the contrast material.

METHOD

The number of our cases is therefore limited. Only those were subjected to bronchographic studies in whom the clinical course of the disease necessitated this procedure. They manifested such symptoms as pulmonary bleeding, persistence of purulent expectoration, and of râles in certain portions of the lungs. The main purpose of taking the bronchograms was to determine the extent of a possible bronchiectasis in order to decide whether or not a partial lobectomy was indicated. The ages of the patients ranged from 28 to 62 years; the duration of the disease from 9 months to 30 years. All patients exhibited evidence of allergy (Table I). Column 4 of the table presents the main findings with which we are concerned, namely the type and extent of bronchiectasis and other bronchographic data.

RESULTS

Only 4 individuals showed definite evidence of irreversible bronchiectasis, namely, 2 of the saccular (Case 2 and 7) and 2 of the cylindrical (Cases 14 and 17) type. In none of the 4 cases did the allergic condition appear to be the cause of the bronchiectasis.

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†Read at the Sixth Annual Meeting of The American Academy of Allergy, Los Angeles, Calif., March 6-8, 1950.

TABLE I

	1		2		3			4			5
					EVIDENCE OF ALLERGY			BRONCHOGRAMS			
CASE NO.	NAME	AGE	DURATION OF ASTHMA (YRS.)	SEVERITY*	IN FAMILY	PERSONALLY (OTHER THAN ASTHMA)†	SKIN REACTIVITY	BRONCHIECTASIS	PSEUDO-BRONCHIECTASIS	DI RIENZO'S CRITERIA	IMPROVEMENT§
1.	Mrs. H. C.	47	1½	++	+	Ecz. N & S	+		+		+++
2.	Mr. A. S.	34	20	++		HF		S‡			–
3.	Mr. K. L.	55	8	+++	+	N & S				+	++
4.	Mrs. M. H.	42	1	+++	+++	HF	+			+	++
5.	Mrs. R. H.	47	1	++	++		++				–
6.	Mrs. Mc C.	40	1	+++			++				++
7.	Mrs. S. R.	33	1	+	+	Ecz.	++	S			+++ (lobectomy)
8.	Mr. T. A.	58	7	+++	+		+		+		–
9.	Mrs. G. C.	30	1	++		N & S	+		+		+++
10.	Mrs. G. S.	44	6	++						+	–
11.	Mr. J. M.	33	20	++					+		+
12.	Mr. E. M.	60	1	++	+	N & S	++		+		–
13.	Mr. E. T.	49	¾	++	+	HF	++				+++
14.	Mrs. J. S.	24	4			N & S		C			–
15.	Mrs. A. R.	49	¾	+	+		++		+		+++
16.	Mr. J. H.	46	2	+++	++	N & S	+				Died (paroxysmal tachycardia)
17.	Miss T. S.	28	3	++	+	G. I. Urtic.	+	C			Died (pneumonia)
18.	Mrs. S. S.	50	5	++	++	N & S	++		+		–
19.	Mr. F. A. Mc	57	3	++	++	G. I.	+				++
20.	Mr. R. O. L.	50	15	++	+++	N & S	++				+
21.	Mrs. D. W. M.	62	30	++	++	N & S	+		+		++
22.	Mrs. I. B.	36	30	+++	++	N & S	+			+	+++
23.	Mr. H. R.	56	10	++	++	HF	+				+++
24.	Mr. Wm. C.	54	20	+++	++	N & S	+++		+	+	+
25.	Mrs. E. C.	49	1			N & S				+	–
26.	Mrs. F. N.	44	4	+++	++		+++			+	+
27.	Mr. T. Mc	56	18								Died (cor pulmonale)
28.	Mr. W. M.	55	20	++		HF	++				–

*+ Little
++ Moderate
+++ Marked

†HF Hay fever
Ecz. Eczema
N&S Nasal and sinus allergy
G.I. Gastrointestinal allergy
Urtic. Urticaria

‡C Cylindrical
S Saccular

§+ No relief
+++ Well

CASE 2.—A. S., a man, was a ragweed-sensitive patient with seasonal asthma. He had a perennial morning cough accompanied by wheezing and productive of considerable purulent expectoration for practically 20 years. Otherwise, he was very little embarrassed physically. The extensive saccular bronchiectasis was confined to one lobe (Fig. 1). The consulting thoracic surgeon believed that the condition was either congenital or had resulted from a pneumonia which he had suffered in early childhood.

CASE 7.—S. R., a woman patient with atopic eczema, had had a tendency to chronic dyspnea and an intractable cough with marked expectoration. Her symptoms were not characteristic of allergic asthma. The infected lobe was removed surgically and the cough and wheezing disappeared.

CASE 14.—H. S., a woman, had cough, wheezing, and dyspnea which occurred only in the morning. This had been initiated by pertussis and was confined to the winter months, being most pronounced on the coldest days of the year. At that time a moderately severe hemoptysis was the usual climax of the condition which yielded to sedatives, rest, and penicillin.

CASE 17.—T. S. had marked dyspnea, associated with expectoration of purulent material, and frequent febrile episodes characterized this condition. The respiratory distress simulated closely that of allergic asthma. The condition was initiated by a bronchopneumonia (Fig 2).

Fig. 1. Fig. 2.

Fig. 1.—Bronchogram showing extensive saccular bronchiectasis in left lower lobe. (Case 2)

Fig. 2.—Cylindrical bronchiectasis of bronchial radicals in left lower lobe. (Case 14)

While each of these 4 individuals exhibited clear evidence of allergy, their wheezing was not typical of that seen in bronchial asthma, with the exception, perhaps of case 17. A large amount of mucus appeared easily with every cough in contrast to the difficulty of expectorating in bronchial asthma.

There were 15 patients in whom no indication of bronchiectasis whatsoever was noted. In the remaining 9, the roentgenologists were not in agreement as to whether or not the findings should be termed bronchiectasis. There

was some widening of secondary bronchi and bronchioles and absence of foliage, suggestive of a "dead tree effect." This was confined to a portion of a lung, usually one or two segments or a lobe, and termed by some "a minimal degree of" or "suggestive of" bronchiectasis. That this lesion had a tendency to disappear is illustrated by the following case report:

CASE 9.—G. C., a 30-year-old woman, was seen on June 11, 1948, having had periodic attacks of bronchial asthma for the past 2 years. They originally started in March and were particularly severe during the grass and ragweed season, at which time hospitalization was required. Neither by history nor by skin testing had sensitivity to any causative agents been detected.

On examination she presented earmarks of allergic asthma, including a positive family background and eosinophilia. The sinuses were clear, the chest showed marked asthmatic wheezing throughout. There was a leucocytosis and an increased sedimentation rate. Intradermal skin tests revealed only minor reactions to some trees, grasses, and such fungi as Monilia, Hormodendron, Torula, and yeast. The patient was asked to eliminate foods which had produced slightly positive reactions. A bronchogram taken September 3 revealed incomplete filling of the bronchi of all lobes in both lungs and some evidence of cylindrical bronchiectasis involving the anterior basal portion of the left lower lobe (Fig. 3).

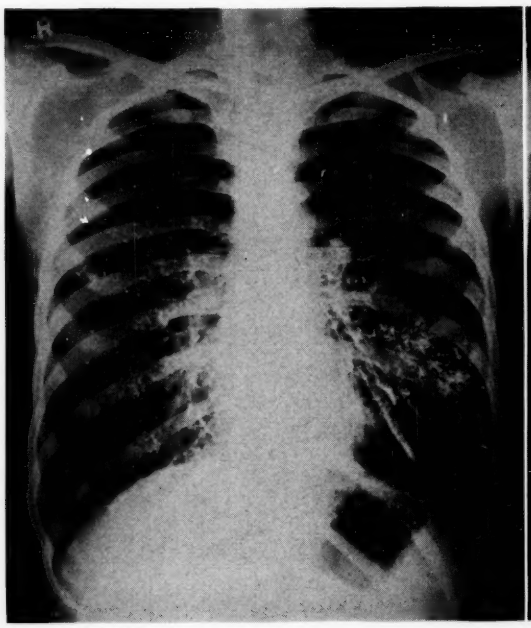

Fig. 3.

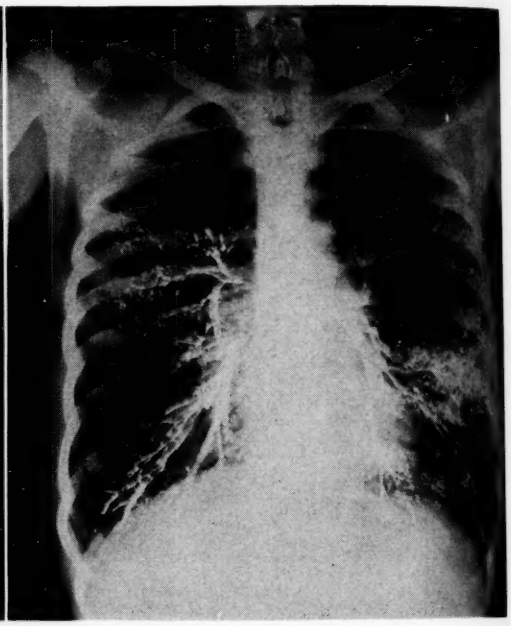

Fig. 4.

Fig. 3.—Pseudobronchiectasis in Case 9. Note the widening of bronchi in basal portion of left lower lobe.

Fig. 4.—Same case 2 months later. Left lower lobe free. Now, similar process in right lower lobe.

As the patient appeared to be refractory to all therapeutic measures, including hyposensitization, antibiotics, and bronchoscopic lavage, she was referred to a chest surgeon in order to consider a partial lobectomy. We felt that this was indicated because of the persistence of the symptoms and because the bronchiectasis was confined to one segment of a lobe. As there was some hesitation on the part of the consultant to perform segmental lobectomy and since the patient insisted on some action, another nationally known chest surgeon was consulted

who repeated the lipiodol studies. At this time the lower right lobe exhibited changes resembling those which had previously been interpreted as bronchiectasis whereas those in the left lobe had completely disappeared (Fig. 4). The surgeon's plan was to wait for one month before proceeding with the partial lobectomy, but the patient's asthma cleared up spontaneously without therapy.

A recurrence in March, 1949, was attributed to trees and grass pollen and this again cleared up spontaneously. She is now completely free from asthma. We refrained from checking the bronchograms as an x-ray film, taken in June, 1949, still revealed residual lipiodol in the lungs.

Changes similar to those seen in this patient were observed in 8 other cases of which 3 recovered completely.

DISCUSSION

In a classical description on serial bronchograms, DiRienzo[4] set up certain criteria for allergic asthma. He believes that in allergic asthma major changes occur in one portion of the lungs, usually in a segment or a lobe. He named the following characteristic features: (1) reduction in caliber of bronchi in one segment or zone of the lungs; (2) absence of fine branching in the terminal bronchi in the affected segment; (3) absence of foliage in this area; (4) delay in progression of the iodized oil into the affected segment; and (5) gas bubbles and mucus in the bronchi. As noted in Section 3 of Column 4 (Table I), 9 cases seem to either fully or partially fulfill these standard requisites. Because no serial bronchographic studies were carried out by us, we are refraining from discussing these findings in detail.

It can be concluded from the survey that irreversible bronchiectasis is very rare in bronchial asthma if existing at all. It was indeed surprising to us that some of the cases with most persistent and severe bronchial asthma failed to show the slightest tendency toward development of bronchiectasis. That the cylindical type of dilation which was noted in 9 cases is a reversible process is indicated by x-ray evidence of Case 9 and by the fact that these patients improved; 3 of them became entirely free from symptoms. In coordinating these observations with DiRienzo's finding, namely, that bronchographic changes are usually limited to a portion of the lungs, it appears likely that the lesions described by us are pathognomonic of allergic asthma. In an autopsy on a patient, not recorded here, the pathologist found that only the bronchi of two pulmonary lobes were filled with thick mucous plugs while the other lobes exhibited only minor changes.

There is a definite need of differentiating these lesions from the true cylindrical and saccular type of bronchiectasis which is of a permanent character. The term pseudobronchiectasis is suggested, thus setting it off as a benign, reversible process for which surgery is not indicated.

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Original Articles

FAILURE TO PREVENT EXPERIMENTAL ECZEMATOUS SENSITIZATION; OBSERVATIONS ON THE "SPONTANEOUS" FLARE-UP PHENOMENON*

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THE examples of poison ivy dermatitis and of allergic eczematous dermatitis due to many causes in industry suffice to point up the exceedingly high incidence and importance of allergic eczematous contact-type sensitization. Therefore, the specific prevention of allergic eczematous sensitization must be considered one of the major goals in dermatologic preventive medicine.

Unfortunately, there is no experimental animal in which an exact replica of the eczematous response, as it occurs in man, can be deliberately produced. Yet all previous work on specific prevention of allergic contact-type sensitization to simple chemicals has been done in experimental animals. The first work dealing with this subject was that of Sulzberger.[1] He observed that intracardial injection of neoarsphenamine in guinea pigs interfered with his attempts to sensitize guinea pigs to neoarsphenamine by the usually sensitizing technique of intracutaneous injection. Later, Kidd and Landsteiner[2] observed that some degree of resistance to sensitization to mustard gas developed in guinea pigs who previously either had inhaled this agent or whose skin had been painted with subsensitizing doses of this agent. Moore[3] reported less convincing work which suggested that the level of mustard gas sensitization developing in guinea pigs can be influenced by the external application of dilutions of mustard gas.

The most important extensive and systematic work on prevention of contact-type sensitization to simple chemicals in guinea pigs has been carried out by Chase.[4] He was able to inhibit susceptibility to cutaneous sensitization with dinitrochlorbenzene to a significant degree by feeding the agent prior to its application to guinea pigs' skin. However, external application of very low nonsensitizing (hereafter called "subsensitizing") doses prior to application of usually sensitizing doses of this simple chemical did not significantly reduce susceptibility to sensitization in guinea pigs. To our knowledge the studies to be reported here are the first attempt at specific prevention of allergic eczematous sensitization in man. Although no decrease in susceptibility to sensitization was observed, it is felt that because of their theoretical importance, the results should be reported.

EXPERIMENTAL

The tests were carried out on 124 hospitalized subjects who were suffering from chronic diseases other than skin diseases. The average age of these sub-

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jects was about 49 years. These patients had had no known previous exposure to 2-4 dinitrochlorbenzene and they were, at the time of the tests, not suffering from any known cutaneous eruption.

The 124 subjects were divided into Group A, consisting of 61 subjects in whom an attempt was made to prevent sensitization, and Group B consisting of 63 subjects who served as controls. The tests can be divided into 4 steps:

Step 1.—The 61 subjects in Group A were "pretreated" as follows: From a tuberculin syringe with a 26-gauge one-inch needle, one droplet of a 1/1000 per cent (1:100,000) solution of recrystallized 2-4 dinitrochlorbenzene (DNCB)* in acetone was dropped on the flexor aspect of the left forearm on each of 23 days between March 8 and April 4, 1949.

Step 2.—The potentially sensitizing exposure was carried out on April 5, 1949, when all 61 subjects in Group A and all 63 subjects in Group B had one droplet of a 10 per cent (1:10) solution of recrystallized DNCB in acetone dropped on the left upper arm from a tuberculin syringe with a 26-gauge one-inch needle.

Step 3.—During the interval from April 5 to May 18, 1949, the sites in all 123 subjects on which the droplet of 10 per cent DNCB had been applied were watched for the occurrence of the flare-up phenomenon.

Step 4.—On May 18, i.e., 44 days after the potentially sensitizing exposure, all 124 subjects were tested for evidence of sensitization by dropping one droplet each of 1 per cent (1:100), 1/10 per cent (1:1000), 1/100 per cent (1:10,000), and 1/1000 per cent (1:100,000) acetone dilutions of DNCB on sites on the flexor aspect of the right forearm of all subjects. The same technique as previously described was used. The reactions were read 48 hours later, i.e., on May 20, 1949.

RESULTS

The results of the tests are presented in Tables I and II.

TABLE I. "SPONTANEOUS" FLARE-UP REACTIONS

	GROUP A (PRETREATED) 61 SUBJECTS	GROUP B (CONTROL) 63 SUBJECTS
Number of subjects with 1 plus flare-up	1	8
Number of subjects with 2 or 3 plus flare-up	22	25
Number of subjects with 4 plus flare-up	7	7
Total number of subjects with flare-up	30	40
Number of subjects without flare-up	31	23

There were 20 subjects who had a definite "spontaneous" flare-up reaction but who subsequently upon testing for sensitization showed no evidence of sensitization. In Group A, 6 subjects evidenced this phenomenon. Of these, 3 previously had a 3 plus flare-up reaction and 3 had a 2 plus flare-up reaction. In Group B, 14 subjects showed this phenomenon. Of these, 1 previously had a 4 plus flare-up reaction; 5, a 3 plus flare-up reaction; 5, a 2 plus flare-up reaction and 3, a 1 plus flare-up reaction. As in previous studies there were also sub-

*Dr. Merrill W. Chase kindly supplied the recrystallized material.

jects who had no flare-up phenomenon but who, upon retesting, were shown to have become sensitized to DNCB, namely, 4 in Group A and none in Group B.

COMMENT

These tests show that in human beings the application to the skin of subsensitizing doses of dinitrochlorbenzene, known to possess a strong sensitizing capacity when applied in 10 per cent concentration, does not reduce susceptibility to subsequent deliberate sensitization to this compound.

TABLE II. SKIN REACTIONS TO DILUTIONS OF DINITROCHLORBENZENE
(44 Days After the Potentially Sensitizing Exposure)

	GROUP A (PRETREATED) 61 SUBJECTS	GROUP B (CONTROL) 63 SUBJECTS
1 plus reactions to 1:100	3	4
2 plus reactions to 1:100	4	3
3 plus reactions to 1:100	14	8
4 plus reactions to 1:100	7	12
1 plus reactions to 1:1000	-	-
2 plus reactions to 1:1000	3	2
3 plus reactions to 1:1000	2	4
4 plus reactions to 1:1000	-	1
Total number of subjects with reactions	28	27
Total number of subjects with no reactions	33	36

This becomes evident from the fact that 28 of the 61 pretreated subjects and 27 of the 63 not pretreated control subjects became sensitized and that there were no significant differences in the levels of sensitivity in the 2 groups. Our results do not exclude the possibility that under different test conditions, e.g., using other concentrations of DNCB or other time intervals of applications, or exposure of larger areas of skin to the subsensitizing solutions, or different routes of administration, different results would have been obtained. However, our results are in agreement with those of Chase[4] in guinea pigs, that external applications are not effective as specific measures for reducing susceptibility to sensitization.

The most interesting finding in the present study was with regard to the "spontaneous" flare-up phenomenon. As will be seen from Table I, there were 30 subjects in the pretreated group and 40 subjects in the control group who showed a spontaneous flare-up. Twenty of those who showed a definite spontaneous flare-up, i.e., 6 in the pretreated group and 14 in the control group, could not be demonstrated to be hypersensitive to DNCB in subsequent skin tests. While this phenomenon has been seen before (for example, Case 3 in Table I in report by Sulzberger and Baer[5]), we were not aware of the extent of its occurrence and we were unable to find any comment about it in the literature. The general impression as to persistence of eczematous sensitivity despite absence of continued exposure to the allergen has been discussed by Sulzberger.[6] Kanof and Rostenberg[7] showed that allergic eczematous sensitivity to poison ivy can persist for many years without exposure to the causative allergen. Wedroff and Dolgoff[8] in their original publication on deliberate sensitization with DNCB

stated that the higher the degree of sensitization the more persistent is the sensitization. But they also found that in all cases which could be observed, the involution of the sensitization occurred spontaneously. Yet they did not report any instance where a "spontaneous" flare-up phenomenon was observed without sensitivity being demonstrable on subsequent testing. Sulzberger and Rostenberg[9] reported that 6 sensitized subjects were retested after 5 to 16 months and that in only 1 of these was there the "spontaneous" loss or reduction of sensitivity described by Wedroff and Dolgoff.

If one accepts the "spontaneous" flare-up in deliberate sensitizations as evidence that sensitization has taken place, and we believe that this is a generally accepted concept (Sulzberger[6] and Grolnick[10]), then our results uncover the fact that among the 55 per cent of subjects who become sensitized there occurs a form of sensitization which develops in about one-sixth of all those exposed to adequate quantities of DNCB, but that this sensitization "spontaneously" subsides to the point where it is no longer demonstrable a few weeks later; this means that while 55 subjects developed a more or less lasting sensitization there were 20 who developed a very short lasting and transitory sensitization. At this rate there would be an additional 39 patients with transitory sensitization for every 100 with more or less permanent sensitization. This sensitization with early "spontaneous" desensitization is perhaps in principle the same phenomenon as that described by Dubois, Schloss, and Anderson[11] many years ago in infants who, after they had ingested a new food, developed for a period of days to weeks to months, a transitory urticarial skin reaction, associated with precipitins in the blood serum, but by no means necessarily accompanied by clinical sensitivity to the particular food; and who then again "spontaneously" lost this urticarial skin sensitivity and the precipitins. And there may be a parallelism between this phenomenon and cases in which allergic urticarial penicillin sensitization can be shown to have been "spontaneously" lost weeks to months after an eruption has occurred. The possibility of a strictly local sensitivity must also be considered as we did not retest our patients at or near the site of original exposure to DNCB. However, Haxthausen[12] was able to produce local sensitization with DNCB only when he exposed an area a few millimeters in diameter; and the exposed sites in our tests were much larger.

It is not possible to state at this time whether the phenomenon of sensitization and early "spontaneous" desensitization is a hitherto overlooked but rather common occurrence in deliberate experimental sensitizations and perhaps also in naturally occurring sensitizations, or whether this is a phenomenon which had its origin in some peculiar feature in our experimental conditions or in the particular group of our test subjects. However, while these subjects were all suffering from chronic disease, most of them were in a generally normal nutritional state since no cachectic patients were included; the age of the test subjects did not seem to play a role, for the phenomenon was seen among young as well as elderly persons. It is certain, however, that if this is a not uncommon phenomenon, it is a phenomenon of the greatest practical importance, particularly with respect to compensation medicine. For if this phenomenon occurs in deliberate eczematous sensitization, then it is very likely to occur in industry and in every-

day life as well. And then, a negative patch test can no longer be considered evidence that an acute allergic eczematous dermatitis which has cleared cannot have been due to an allergen which several weeks later did not elicit a positive patch test.

SUMMARY

1. An attempt was made in human subjects to specifically lessen their susceptibility to eczematous contact-type sensitization with dinitrochlorbenzene. This was done by applying to their skin subsensitizing doses of this agent for several weeks prior to exposure to a quantity of this agent, which is known frequently to incite sensitization.

2. No diminution was noted in the capacity of such subjects to become sensitized to dinitrochlorbenzene as compared with a not pretreated control group.

3. Seventy of 124 subjects exposed to the potentially sensitizing doses of dinitrochlorbenzene developed a "spontaneous" flare-up, but several weeks later 20 of these could no longer be shown to be hypersensitive to this compound.

4. It appears possible that sensitization followed by early "spontaneous" desensitization may not be an uncommon immunologic phenomenon in allergic eczematous sensitization.

We are indebted to Drs. Henrik M. C. Luykx and Merrill W. Chase for their advice in the planning of these tests.

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CARDIORESPIRATORY FUNCTION IN RELATION TO ASTHMA AND EMPHYSEMA*

Viewpoint of the Allergist

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WE ARE greatly indebted for much of our recent knowledge of respiratory function to Dr. Cournand, Dr. Richards, and their associates.[1] One of the early organizations in this country of clinical allergists was known as The Society for the Study of Bronchial Asthma and Allied Conditions. This serves to emphasize the fact that although known as allergists we have a broad interest in the physiology and pathology of the respiratory tract, especially as it relates to bronchial asthma and its complications. I shall try to indicate briefly several ways in which this work is important to us as clinical allergists with a real interest in all phases of bronchial asthma.

In the first place it must be emphasized that at the present time respiratory function tests serve to increase our knowledge of the physiology of respiration rather than offer to us techniques for use in our daily management of patients. Very few hospitals are as yet equipped with the complicated apparatus necessary for thorough study of cases along the lines which Dr. Cournand and Dr. Richards have spoken of, and certainly there are very few men available as yet who have the technical skill and training necessary for conducting these tests properly.

Therefore, the important thing at present is for us to understand what this work means to us in a better understanding of bronchial asthma and its complications, to offer our patients for study by groups qualified in cardiorespiratory function, and through our experience in the clinical problems involved to try and give impetus to further work along lines which we believe will add further to better understanding and management of the patient with bronchial asthma.

One thing that stands out in this work which is of paramount importance is the concept of progressive pulmonary insufficiency. We have just seen a striking example of this at the New York Hospital Allergy Clinic.

CASE REPORT

The patient is a Negro man 37 years of age who has had asthma since 3 years of age. He complains of recurrent paroxysms of asthma and dyspnea which for the last two years have occurred on the slightest effort. Physical examination shows a young-appearing male, breathing accelerated, marked barrel chest, wheezy râles, large liver, and swollen ankles. From an allergic standpoint he reacts markedly to ragweed pollen, house dust, and feathers. Roentgenograms of his nasal sinuses show cloudy antra. Examination of the nose shows an allergic type of membrane with no definite pus. Roentgenogram of the chest shows hyperaeration, low fixation of the diaphragm, marked accentuation of peribronchial markings, and moderate enlargement of heart. The blood pressure is elevated. Wassermann is negative. Complete blood count shows polycythemia. The urine is concentrated with a 3 plus albumin. Electrocardiogram indicates right axis deviation.

*Presented at a symposium on "Cardio-Respiratory Function in Relation to Asthma and Emphysema" at The New York Allergy Society, November 2, 1949. Dr. Andre Cournand spoke on "Physiologic Considerations" and Dr. Dickinson W. Richards, Jr. on "Viewpoint of the Internist."

Cardiopulmonary function tests were done on him by Drs. C. Dotter and D. S. Lukas who are in charge of the laboratory recently set up at The New York Hospital for these studies. Some of the results are condensed as follows:

Vital capacity, observed 2,202 c.c., predicted 4,013 c.c. Residual air, observed 4,237 c.c., predicted 1,226 c.c. Total capacity, observed 6,439 c.c., predicted 5,234 c.c. Maximum breathing capacity in L/min. 24.9, predicted 116.3. Breathing reserve in per cent, basal 72 per cent, predicted 92 per cent. Index of intrapulmonary mixing: alveolar N2 per cent 10.2, predicted (i.e., less than) 2.5 per cent. Oxyhemoglobin saturation in per cent of arterial blood, basal 69.6, predicted 96.2. Carbon dioxide content in vols. per cent, basal 69.4, predicted 52. Oxygen consumption in c.c./min./ m2 basal 109, predicted 131; oxygen removal in c.c./L of ventilation, basal 27, predicted 46. Pulmonary arterial pressure in mm. Hg 73/42 (normal 25/8).

In their summary of this case Drs. Dotter and Lukas state, "From the functional standpoint there is considerable evidence of gross impairment of the patient's ventilatory capacity. The maximum breathing capacity, or the maximal volume of air that can be moved in and out of the lungs per unit time with the patient selecting his own rate and tidal volume, is only 24.9 L/min. or 21 per cent of the predicted value. The factors that tend to make for the patient's severe ventilatory insufficiency are many, but chiefly is the loss of elasticity of the pulmonary parenchyma and its secondary effects on the rib cage with maintenance of this structure in the inspiratory position. In addition to these restrictive factors, however, there is evidence of the operation of a chronic obstructive factor of a bronchospastic nature. Thus, maximal breathing is performed in the extreme inspiratory position. Following an injection of epinephrine 1:1000 solution the expiratory phase became less prolonged, maximal breathing was performed at a lower inspiratory level, the vital capacity was increased by 27 per cent, and the maximum breathing capacity was increased by 30 per cent as compared to prebronchodilator values.

"Also severe disorder of the alveolorespiratory component of pulmonary function is indicated. The high index of intrapulmonary mixing (i.e., the per cent nitrogen remaining in alveolar air after seven minutes of pure oxygen breathing) indicates inefficient and unequal distribution of inspired air in the alveoli with resultant underventilation of large number of alveoli. Alveolar underventilation is further indicated by the significantly higher than normal arterial blood CO_2 content. The striking arterial oxyhemoglobin unsaturation is probably due to perfusion of underventilated alveoli and perhaps to thickening of the alveolocapillary membrane by fibrosis secondary to the basic pulmonary pathologic process and the cardiac failure. The basal pulmonary arterial pressure here of 73/42 contrasts markedly with the normal value of 25/8."

All this is rather technical but it certainly serves to emphasize the point that here in an asthmatic, 37 years of age, with asthma since the age of 3, there has been progressive respiratory insufficiency which has finally culminated in an extreme degree of cardiopulmonary failure.

Investigation of alveolocapillary exchange of oxygen and carbon dioxide indicates that when pulmonary circulation is maintained in partially aerated

alveoli, or when the diffusion through the alveolar membrane is reduced as a result of thickening, or fibrosis of the alveolar wall, or a coating of fluid on the alveolar surface, anoxemia begins to be a factor which may have profound effects. The longer bronchial asthma is allowed to progress the greater will be the degree of impairment in maximum breathing capacity and maximum ventilatory capacity per minute and inevitably the patient with bronchial asthma does not have normal capacity for effort without dyspnea. Along the same line these studies in less severe cases indicate the development of emphysema as a complication of bronchial asthma long before the barrel chest, obvious cyanosis, increased areas of pulmonary resonance, and myocardial damage are apparent. We are learning from these tests even in early emphysema where there is a fairly normal vital capacity and normal oxygen saturation and no manifest cyanosis that there may be a marked handicap in respect to absorption of oxygen into the arterial blood necessitating a burdensome increase in pulmonary ventilation to prevent anoxia with resultant fatigue on the part of the patient. In a word, many of our chronic asthmatics who complain of fatigue without obvious emphysema or cardiac signs are truly tired out attempting to maintain their normal oxygen saturation by increased respiratory effort.

As pulmonary insufficiency progresses due to emphysema and fibrosis, increased pressure in the pulmonary artery puts more strain on the right side of the heart, anoxia becomes demonstrable by analysis of the blood for oxygen saturation, and we have our typical case of bronchial asthma with emphysema suffering from both cardiac and pulmonary insufficiency with a degree of anoxia which interferes seriously with cerebration and even the minimal effort of maintaining basal metabolic function.

In our management of status asthmaticus where anoxia is such an important factor, these tests and a better understanding of cardiorespiratory function may well offer to us great improvements. The variety of measures available for administering oxygen will have to be checked by these tests. Certainly there is considerable confusion in most hospitals today concerning the proper method of oxygen administration and this applies not only to the various types of apparatus but also to questions of oxygen concentration and the degree of pressure. Furthermore, how important helium may be and under what conditions its use is obligatory is another question far from settled in our clinical management of status asthmaticus. Further testing of patients with status asthmaticus will help to demonstrate that in cases of anoxemia excessive sedation may depress respiratory function and lead to further anoxemia and even acidosis. Our whole armamentarium of drugs to relieve bronchial spasm will have to demonstrate through these tests their relative efficiency to do the job for which claims are made. It would seem also likely that these tests for respiratory function may add greatly to our understanding of the influence of climatic and atmospheric changes in respect to the symptoms of the asthmatic who is daily measuring his comfort by whether it is wet, dry or humid, hot or cold, the atmospheric pressure high or low. The day may come when we shall have objective tests for telling a patient whether the climate of Arizona, South-

ern California, Florida, or even New York City is best for him, and certainly the effectiveness of air conditioning methods will have to be judged in the light of information derived from these means.

In concluding, it should be emphasized that bronchial asthma is not a simple reversible process, but that, if continued, changes take place in the lungs which are irreversible and lead to impaired oxygenation of the blood, increased pulmonary arterial tension with involvement of the myocardium, and a diminution of maximum breathing capacity with poor or impaired tolerance of activity. Most important for us as allergists is that we emphasize the thorough study of each bronchial asthmatic with the aim of eliminating as many causes of the asthma as early as possible. We must emphasize the reality of sensitization as the cause of much chronic asthma and the means we have for treatment and elimination. We must not neglect the importance of upper respiratory tract infection involving the nasal sinuses and infected lymphoid tissue in the nasopharynx. We must be alert to the importance of intercurrent bronchial and pulmonary infection. We should be mindful of the complications of cardiac insufficiency which follow in the train of respiratory insufficiency. We should combat with all force the idea that asthma in children is an inconsequential ailment that the child will outgrow without the necessary allergic tests and other management to clean up the asthma as soon as possible. Every day we hear of some new palliative drug, some compromise in therapy that will moderate asthma without removing the cause. This is dangerous doctrine that will in many asthmatics only delay but not prevent the serious irreversible changes in the heart and lungs in late middle life that plague so many asthmatics who have been treated in this expectant fashion.

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NEWER ANTIHISTAMINICS: III. SOME PHARMACOLOGIC AND THERAPEUTIC EFFECTS OF β-(P-METHYLBENZHYDRYLOXY)-ETHYLDIMETHYLAMINE HYDROCHLORIDE, A DERIVATIVE OF DIPHENHYDRAMINE HYDROCHLORIDE*†

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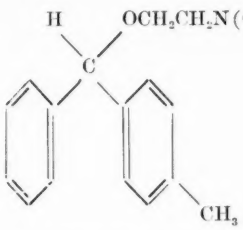
IN NORMAL human subjects and in those suffering from allergic diseases we have previously reported the effects of diphenhydramine hydrochloride (Benadryl),[2, 7-10, 13-18, 20-23] and of β-morpholino-ethyl benzhydryl ether hydrochloride (Linadryl).[19] Of these, diphenhydramine hydrochloride, despite the relatively high incidence of side reactions, especially somnolence, has proved to be the more effective and more widely applicable to allergic disease. The present report is concerned with a summary of our investigations of another derivative of diphenhydramine hydrochloride, which varies chemically from the parent substance by the presence of a methyl grouping on one of the phenyl rings. Structurally, as noted below, it is β-(p-methylbenzhydryloxy)-ethyldimethylamine hydrochloride and may be called methyl diphenhydramine hydrochloride, or for the sake of brevity, Toladryl.



Molecular weight = 305.84

β-(p-methylbenzhydryloxy)-ethyldimethylamine hydrochloride

In animals, Toladryl is reported to be a much more powerful antihistaminic than diphenhydramine hydrochloride.[12] It is from two to four times as effective as the latter in protecting guinea pigs from lethal doses of histamine aerosols. However, its antiacetylcholine or atropine-like effect is only one-tenth that of diphenhydramine. The two drugs are equally effective in counteracting the smooth muscle spasm produced by barium ("myotropic" antispasmodic activity), and neither augments the rise in blood pressure produced by epinephrine. When intravenously administered to rats, the LD-50 of Toladryl is approximately the same as that for diphenhydramine hydrochloride, and in short term studies of chronic toxicity, there is little difference between the two in their effects upon growth and gain in weight.

The purpose of the present report is (1) to record such pharmacological data as was obtained by the administration of the drug to normal individuals

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†Generous supplies of this material have been made available through Dr. E. A. Sharp, Director of Clinical Investigation, Parke, Davis & Co., Detroit, Mich., whose courtesy is herewith gratefully acknowledged.

and to patients, (2) to evaluate the range of activity of the drug, (3) to determine the toxicity or unpleasant side effects produced by the drug, and (4) to compare these findings with those obtained after the administration of diphenhydramine hydrochloride under similar conditions.

METHODS AND MATERIALS

Approximately 131 normal persons and 205 patients have been the subjects of these observations. By normal subjects are meant outpatients or ambulatory ward patients whose medical conditions are of such degree and character as not to influence observations on the type of drug under consideration. Of the normal subjects, 54 have been on a standardized routine of living described elsewhere.[7] The remainder have been used for one or more of the special pharmacological problems, such as the effects of the drug on the eye, glucose tolerance, gastric acidity, basal metabolic rate, electrocardiogram, liver function, hematopoiesis, weight, and water balance. Signs of toxicity have been watched for in all subjects.

In the clinical evaluation of the drug, the observations of the several investigators have been obtained independently but the technique of recording data has been the same for all members of the group and the grading of results has been reduced to a uniform basis. Details of histories, physical examinations, and laboratory tests have been designed to afford a thorough appraisal of the status of important bodily functions. Patients were seen once weekly or oftener, and the detailed accounts of their symptoms were fully appraised. In order to facilitate the work, each patient was furnished with cards so arranged as to allow him to record variations in dosage, changes in old symptoms, and the appearance and severity of new ones. Where doubt existed as to the validity of the observations made by the subjects, placebos were also employed. Moreover, they were used in all subjects who demonstrated dramatic changes for the better following the use of the antihistaminic compound. In recording the data, we have used the terms "complete relief," "partial relief," and "no relief" to designate the disappearance of all symptoms, the loss of more than 50 per cent of distress, and little or no improvement, respectively. Unless otherwise specified, all the observations here recorded have been made following oral administration of the drug in doses of from 150 to 600 mg. daily given in divided doses 3 times daily. Effects upon the eye were recorded following topical application. Intravenous glucose tolerance tests were done according to the method of Thorn, Koepf, Lewis, and Olsen[25] with modifications as previously described.[22] Methods for other laboratory procedures are the same as were used in our investigations of diphenhydramine and are given in detail elsewhere.[16, 18] The techniques employed in eliciting dermal responses and in determining ophthalmic effects have been previously described.[7, 8]

RESULTS

I. PHARMACOLOGICAL DATA

1. *The Antihistamine Effects*

A. *Supression of the dermal response to histamine* (Table I and Fig. 1).—The wheal and flare responses of 30 normal subjects were recorded following the

oral administration of Toladryl in doses varying from 150 to 600 mg. daily over periods of from one to 8 weeks. Decrease in the size of the wheals produced by the standard technique previously described was consistent with an increase in dose for all patients, while the flare reactions showed considerable variation, although a similar downward trend in the area involved was obvious. If we compare the influence of this drug upon the wheal and flare reactions with that of diphenhydramine hydrochloride,[2, 18] we will note that, weight for weight, the activity is approximately 50 per cent of that for the drug previously studied.

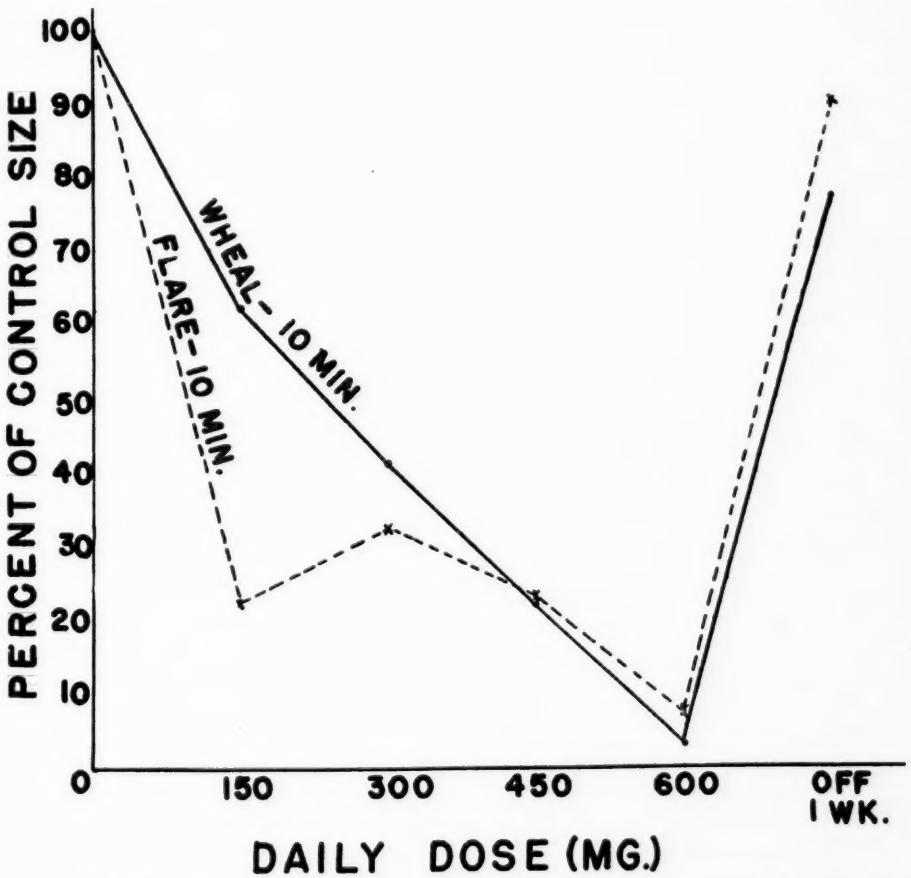

Fig. 1.—Wheal and flare reactions to intracutaneously administered histamine at 10 minutes in 30 subjects given Toladryl in doses noted for periods of not less than one week at any given level of dosage.

In the "release" phase, the reaction of the patient to histamine is still slightly depressed at the end of one week after discontinuance of the drug in doses of 600 mg. daily. In this, again, the present compound resembles diphenhydramine hydrochloride, although with the latter, the depression at the end of one week is greater than for the newer preparation.

B. *The Effect upon gastric acidity* (Table II).—In 11 "normal" subjects, fractional gastric analyses were performed before and immediately after one

week of medication with Toladryl in daily doses of 300, 450 and 600 mg., respectively. In 5 of these subjects, there was no free hydrochloric acid present at any time, neither before nor after histamine, nor while taking Toladryl. While slight individual variations in the figures for combined gastric acid occurred under the influence of Toladryl in such subjects, these were not statistically significant. In one instance, free hydrochloric acid appeared in the gastric juice one week after the drug had been discontinued.

TABLE I. WHEAL AND FLARE REACTIONS TO INTRACUTANEOUSLY ADMINISTERED HISTAMINE FOLLOWING THERAPY WITH β-(P-METHYLBENZHYDRYLOXY)-ETHYL-DIMETHYLAMINE HYDROCHLORIDE

	MEDICATION			RESULTS EXPRESSED IN TERMS OF PERCENTAGE CHANGE FROM CONTROL					
				WHEAL			FLARE		
NO. OF SUBJECTS	DAILY DOSE (MG.)	PERIOD (WKS.) RANGE	PERIOD (WKS.) AVERAGE	TIME (MIN.)	RANGE	AVERAGE	TIME (MIN.)	RANGE	AVERAGE
9	150	1-4	2.5	5	0-400	62	5	0-116	31
				10	0-209	62	10	0-175	22
				15	0-147	55	15	0-111	27
7	150	4	4.0	5	0-100	29	5	0-58	12
				10	0-75	33	10	0-20	3
				15	0-71	33	15	0-29	6
15	300	1-5	1.5	5	0-150	50	5	0-132	49
				10	0-125	41	10	0-86	32
				15	0-85	27	15	0-91	34
16	450	1-8	2.6	5	0-200	44	5	0-58	17
				10	0-67	22	10	0-93	23
				15	0-71	22	15	0-68	17
7*	600	1	1.0	5	0-57	16	5	0-70	22
				10	0-20	3.4	10	0-26	7.4
				15	0-37	5.3	15	0-10	7.3
5	Off for	–	–	5	0-134	83	5	18-240	118
Total 59	1 week			10	0-150	77	10	0-230	90
				15	0-250	99	15	0-165	71

*These subjects were successively treated with 300, 450, and 600 mg., for not less than one week at each level of dosage.

In the 6 subjects who showed free hydrochloric acid in the "fore" or "control" gastric analyses, there was a tendency for this to be increased following the use of Toladryl at all levels of dosage (Table II), but this change was not statistically significant until 600 mg. of drug daily had been employed for one week (Table II). However, even at this level of dosage the individual variations were wide, as 2 of the 6 subjects exhibited values below those of their control readings throughout the test.

It is clear that neither diphenhydramine hydrochloride nor Toladryl exerts a predictable action upon gastric secretion, but in the former instance the trend is toward depression[15, 18] while in the latter there is no significant deviation except in the largest doses, under which circumstances the free but not the combined acid is significantly increased.

2. *The Atropine-like Activity (Fig. 2)*

Despite the relatively poor antiacetylcholine activity observed for Toladryl upon the tissues of small laboratory animals and on isolated tissue from them, namely, one-tenth that for diphenhydramine hydrochloride,[24] its action upon the

human eye was studied following its oral and topical administration, respectively. When taken orally in daily doses ranging from 150 to 600 mg. for periods varying from 3 to 6 weeks, there was no variation in the size of the pupil in the 30 subjects studied. Subjective phenomena suggesting disturbances of vision have likewise been absent following oral ingestion, even in the largest doses. In this regard the drug differs from diphenhydramine hydrochloride with which blurring of vision, probably indicating interference with accommodation, has been present in about 4 per cent of all cases.[18]

TABLE II. AVERAGED VARIATIONS IN SECRETION OF FREE HCL BY GASTRIC MUCOSA FOLLOWING ADMINISTRATION OF TOLADRYL TO "NORMAL" SUBJECTS

NUMBER OF SUBJECTS	6	6	6	6
DOSAGE (MG. DAILY)	0	300	450	600
DURATION OF TREATMENT (WEEKS)	–	1	1	1
Free HCL* in				
Fasting Specimen	+1.4*	+1.6†	−0.6†	+0.8†
15 min. Specimen	+4.5	+7.5	+3.0	+7.8
30 min. Specimen	+11.2	−2.2	+3.6	+23.8
45 min. Specimen	+16.2	−1.9	+4.6	+19.8
60 min. Specimen	+12.5	+1.5	+6.7	+18.9
75 min. Specimen	8.2	+1.8	+2.2	+18.6
90 min. Specimen after histamine injection	+24.6	+2.4	+5.8	+39.2

*Expressed in degrees, each equivalent to 0.00365 Gm. hydrochloric acid.

†Expressed in degrees of hydrochloric acid as a variation above (+) or below (−) the control value.

When Toladryl was applied topically to the eye in aqueous solutions of 0.5 per cent, its action, although much weaker, imitated qualitatively that of diphenhydramine.[9, 18] Our results have been described in detail elsewhere[1] and the methods of investigation are the same as those employed in the earlier testing of diphenhydramine hydrochloride.[9] Following the instillation of a 0.5 per cent solution into the eyes of 60 subjects, we found pupillary dilatation in 40, and no change in 20. Visual acuity appeared to be improved in 10 subjects, diminished in 10, and unaltered in the remaining 40. The ability to accommodate was decreased in 36 individuals and unchanged in 24.

In 15 subjects, the effects of Toladryl alone and of Methadryl in combination with epinephrine (0.001 per cent), homatropine (1 per cent) and eserine (0.5 per cent), respectively, were compared (Fig. 2). The characteristic effects of each of these agents were recorded following its topical application, and one hour later, 0.5 per cent aqueous solution of Toladryl was instilled into the eye. Fifteen minutes thereafter the pupillary size was again determined and the chart (Fig. 2) has been constructed from data thus obtained. When Toladryl was instilled following such use of epinephrine there was a summation of the very weak mydriatic effect of the two drugs. There was a similar augmentation of action when homatropine and Toladryl were employed. Contrariwise, Toladryl perceptibly antagonized the miotic action of eserine.

In view of all these facts, it appears that Toladryl exerts a slight, but measurable, atropine-like effect upon the eye when topically applied.

3. *Other Actions*

A. *Central nervous system.*—The influence of Toladryl upon the central nervous system will be further touched upon in connection with toxic reactions (q.v.). Toxic reactions of any kind were much less frequently seen than with diphenhydramine hydrochloride, and whenever they occurred were much less severe in degree. However, at the higher levels of dosage, Toladryl in several instances caused a variety of cerebral symptoms ranging from insomnia and agitation to disorientation (450 mg. daily or more).

B. *Cardiovascular system.*—Toladryl produced no disturbances in pulse rate or in blood pressure in doses ranging from 150 to 600 mg. daily. The postural hypotension which was seen when larger doses of diphenhydramine hydrochloride were used did not occur during the administration of Toladryl.

Electrocardiograms were obtained from all normal subjects weekly and from a majority of patients before and during therapy with Toladryl. No changes were observed as a result of doses as high as 600 mg. daily continued for as much as 8 weeks in subjects who had previously used the drug at some dosage level for an additional 6 weeks.

C. *Glucose tolerance* (Table III).—Following the administration of Toladryl for periods of time ranging from one to several weeks, intravenous glucose tolerance curves were completed in 11 subjects under standard conditions already detailed elsewhere.[9] (Table III.) Six patients showed rather marked improvement in glucose tolerance, 2 showed slight improvement, and 3 a somewhat diminished tolerance. Improvement was most striking after 450 mg. of the drug daily had been employed for one week. At this level of dosage, it appears that the depression of the fasting, 1- and 2-hour values may be statistically significant, as in each instance the value for P is slightly less than 0.05. None of the other alterations in blood sugar following the use of Methadryl in any dose appear to be of importance.

D. *Basal metabolism* (Table IV).—The basal metabolic rate was determined serially in each of 14 subjects to whom Toladryl was given in gradually increasing doses, ranging from 150 to 600 mg. daily. The data, summarized in Table IV, indicate that Toladryl may lower the basal metabolism slightly. That such results are not due to the gradually increasing familiarity with the basal metabolism machine and a consequently diminishing fear of each succeeding test does not seem likely, as these subjects had had numerous such tests under similar conditions and in the same surroundings prior to their participation in this study.

E. *Fasting blood chemical determinations* (Table V).—In 17 subjects, efforts were made to follow hepatic function by means of serial determinations of the icteric index, van den Bergh reaction, cephalin cholesterol flocculation, and serum phosphorus, phosphatase, cholesterol, and its esters, and total proteins with albumin and globulin fractions. The results, recorded in Table V, show variation in all values, as well as the individual and group averages to be within the range of normal during the administration of Toladryl in daily doses which varied from 150 to 600 mg. over periods of time which extended from 3 to 14 weeks with an average of 7.3 weeks.

In 32 subjects, values for urea nitrogen, creatinine, and glucose of the blood remained well within normal limits throughout periods of treatment ranging from 4 to 12 weeks with gradually increasing daily doses of Toladryl, varying from 150 to 600 mg. (Table V). Throughout the study these values were not significantly altered from the normal figures obtained during the control period.

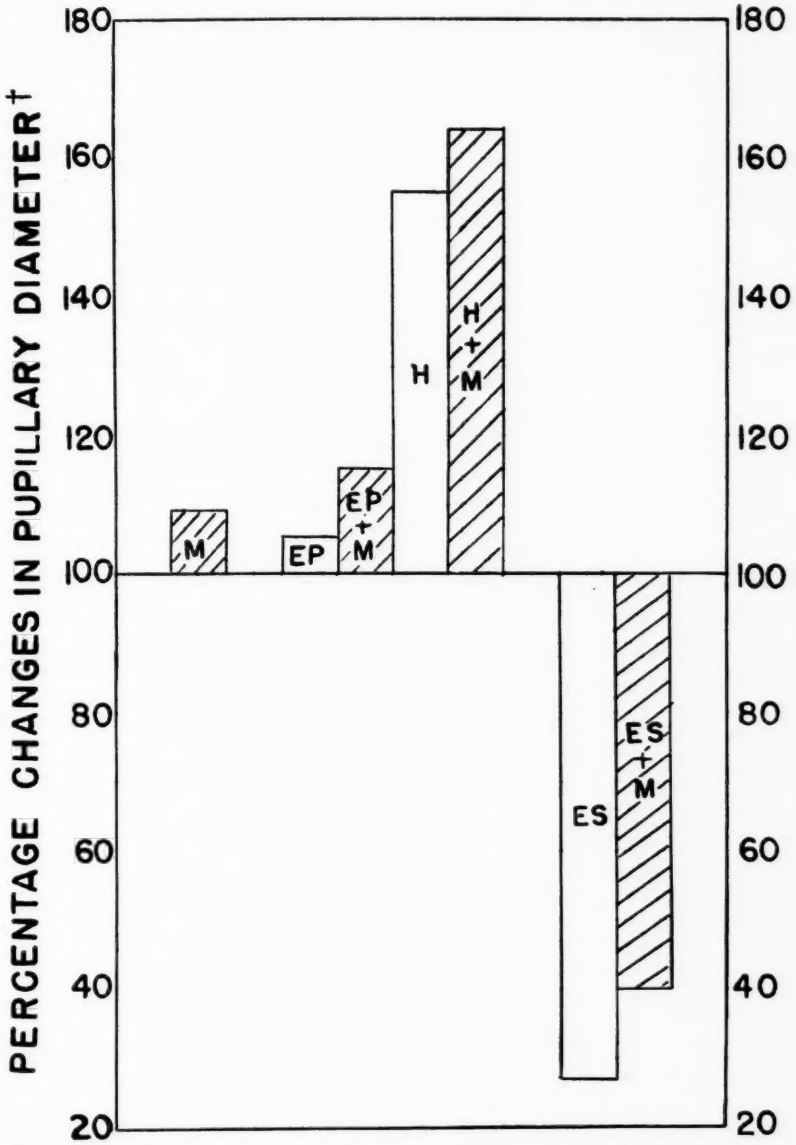

Fig. 2.—Changes in the pupil of the human eye following topical applications* (average for 15 subjects).

M, Toladryl (0.5 per cent); *EP*, epinephrine (0.001 per cent); *H*, homatropine (1.0 per cent); *ES*, eserine (0.5 per cent).

*The "fore" measurement is arbitrarily accepted as 100 per cent in terms of which all subsequent readings are calculated.

†Indicates that Toladryl was applied following the initially mentioned drug.

TABLE III. INTRAVENOUS GLUCOSE TOLERANCE CURVES IN ELEVEN SUBJECTS BEFORE AND AFTER RECEIVING TOLADRYL

NO. TESTS	DOSE DAILY (MG.)	DURATION OF TREATMENT (WKS.)	AVERAGED LEVELS FOR BLOOD SUGAR AT THE HOURS INDICATED													
			MG. PER 100 C.C.							CALCULATED AS PER CENT OF CONTROL FIGURE*						
			0	1	2	3	4	5	6	F†	1	2	3	4	5	6
11	Control	1	127.3	338.1	227.8	153.3	124.9	111.4	101.9	100.0	100.0	100.0	100.0	100.0	100.0	100.0
16	300	1	107.6	327.2	205.8	144.4	112.0	91.6	95.8	87.8	91.8	93.4	97.0	93.7	86.0	102.4
11	450	1	101.9	274.7	182.0	124.3	106.7	98.9	92.8	84.1	88.1	85.4	87.6	89.6	96.6	93.4
12	600	1	110.4	310.3	208.5	145.7	121.2	106.4	115.8	86.3	89.4	97.0	97.4	100.2	98.4	119.6
3	off 600	1	107.3	366.4	218.6	173.7	116.3	113.0	100.0	85.0	104.3	86.0	98.7	85.3	91.3	96.3

*Control figures are arbitrarily given a value of 100 in making these calculations.

TABLE IV. ALTERATIONS IN BASAL METABOLIC READINGS BEFORE AND DURING ADMINISTRATION OF TOLADRYL

TREATMENT		CASES				B.M.R. (NO.)	(B.M.R.—PER CENT)		
DAILY (MG.)	DURATION (WKS.)	TOTAL	AS COMPARED WITH CONTROL, B.M.R. WAS:				VARIATION FROM CONTROL		
			INCREASED	UNCHANGED	DECREASED		RANGE	AVERAGE	P VALUE
0	0	14	–	–	–	34	–10 to +25*	+ 6.8*	–
150	4.3	3	1	0	2	11	–25 to + 9	– 1.4	–
300	1.5	14	5	0	9	28	–31 to +19	–10.4	0.01
450	1.9	13	2	1	10	26	–31 to + 7	–13.4	<0.02
600	1.0	9	4	1	4	12	–39 to +17	– 7.4	<0.2

*Control figures represent actual values from which subsequent variations were determined in percentage.

TABLE V. MISCELLANEOUS CHEMICAL DETERMINATIONS UPON BLOOD OF NORMAL SUBJECTS RECEIVING TOLADRYL

DOSE (MG. DAILY)	0		150		300		450		600	
NUMBER OF SUBJECTS	17		5		17		11		9	
DURATION TREATMENT (WKS.)			1-8		1-2		1-2		1-2	
AVERAGE DURATION			3.3		1.2		1.3		1.5	
	RANGE	AVG.	RANGE	AVG.	RANGE	AVG.	RANGE	AVG.	RANGE	AVG.
Icterus index	4.0-10.0	4.9	4.0-5.0	4.0	4.0-100	7.1	4.0-150	5.8	4.0-13.0	4.8
van den Bergh	neg.	neg.	neg.-tr.	neg.	neg.-tr.	neg.	neg.-1+	neg.	neg.-1+	neg.
Cephalin flocculation	neg-4+	1.1+	neg-2+	neg.	neg.-4+	1.8+	0-4+	2.1+	neg.-3+	1.8+
Phosphorus mg. %	3-5.2	3.6	3.6-3.8	3.7	3.0-5.2	4.5	3.1-4.5	3.6	3.2-4.5	4.0
Phosphatase units	1.6-12.0	4.0	3.2-5.3	4.5	2.7-11.6	4.8	2.3-11.6	3.8	2.4-10.0	4.1
Total cholesterol mg. %	130-344	224	214-250	234	148-440	255	126-296	240	156-330	245
Cholesterol esters	80-250	148	129-169	155	110-275	172	72-211	162	101-246	170
Total proteins Gm. %	5.8-7.9	7.1	7.0-7.5	7.1	7.2-8.6	7.3	6.6-8.3	7.5	5.7-8.3	7.2
Albumin	3.0-7.0	4.9	4.7-5.5	4.9	4.4-5.3	4.7	4.3-5.3	4.8	4.0-5.4	4.5
Globulin	1.7-2.9	2.2	2.2-2.8	2.4	2.4-3.3	2.6	2.0-2.9	2.7	1.5-3.4	2.6
A/G	1.5-3.0	2.2	2.0-2.2	2.1	1.5-2.3	1.8	1.6-2.4	1.8	1.3-2.8	2.2
NUMBER OF SUBJECTS	32		22		26		12		10	
DURATION TREATMENT (WKS.)			1-6		1-2		1-2		1-2	
AVERAGE DURATION			1.5		1.1		1.3		1.6	
Urea nitrogen mg. %	8.7-23	13.2	8.5-15	10.7	8.7-12.5	9.5	8.0-20.0	11.3	8.5-13.0	14.5
Creatinine mg. %	0.9-1.8	1.1	0.9-1.2	1.0	0.9-1.0	0.9	0.9-1.3	1.0	0.9-1.2	1.0
Blood sugar mg. %	60-120	95	68-110	83	80-97	92	85-110	95	85-120	108

TABLE VI. INFLUENCE OF TOLADRYL UPON THE HEMOGRAMS OF NORMAL SUBJECTS

NUMBER OF SUBJECTS	37		44		37		16		10	
DURATION TREATMENT (WKS.)			1-8		1-8		1-7		1-2	
AVERAGE DURATION			2.5		1.8		1.9		1.6	
	RANGE	AVG.	RANGE	AVG.	RANGE	AVG.	RANGE	AVG.	RANGE	AVG.
Hemoglobin Gm. %	8.5-17.0	14	10.0-17.0	14	8-16.5	15.9	7.0-18.5	13.5	7.5-16.0	14.4
Red blood cells (millions)	3.38-4.99	4.26	3.05-4.88	4.10	2.89-4.55	4.34	2.87-4.98	4.0	3.07-4.7	4.2
White blood cells (thousands)	4.25-15.8	7.6	5.0-10.3	7.15	5.01-11.4	7.42	5.45-11.3	7.82	5.87-8.75	6.85
Polymorphonuclears %	26-79	58	43-81	55	32-79	60	30-79	60	42-78	62
Lymphocytes %	20-72	41	23-50	38	18-65	35	20-66	32	20-56	36
Monocytes %	0-8	2.4	0-6	2.8	0-5	2.2	0-6	2.8	1-3	1.4
Eosinophiles %	0-4	0.7	0-4	0.8	0-6	1.5	0-2	2.1	0-3	1.0
Basophiles %	0-1	0.1	0-1	0.01	0-1	0.11	0	0	0	0
Erythrocyte sed. rate 15/60 min.	0/5-6/82	2/25	-	-	-	-	-	-	4/17-2/20	3/18
Hematocrit %	23-58	35	-	-	-	-	-	-	34-36	35

F. *Hemograms.*—Blood counts, hematocrit readings, hemoglobin determinations, and the speed of sedimentation of erythrocytes were determined in 37 normal subjects before and after Toladryl was given in doses of 150, 300, 450, and 600 mg. daily, respectively, for total periods ranging from 4 to 25 weeks with an average period of treatment, including all levels of dosage, of 7.8 weeks (Table VI). While slight deviations from control values were encountered in the hematocrit readings and sedimentation times, there were no statistically significant changes from normal in any of the data obtained.

G. *Urinalyses.*—Urinalyses were made weekly in all hospitalized individuals (61) to whom Toladryl was given orally at any level of dosage. In no instance, was there a significant change in our findings between periods of control and those of therapy.

H. *Weight and water balance.*—The precise determination of water balance was not attempted in connection with administration of Toladryl. However, throughout the study, subjects received a standardized diet, were carefully checked in regard to fluid intake and urinary output, and were weighed at the same hour daily on scales with an accuracy of plus or minus 7.5 grams for all weights between 1.0 and 150.0 kilograms. Data so collected revealed no alterations in body weight or water balance in any of the subjects, who were treated over periods of time ranging from 3 to 13 weeks.

II. THERAPEUTIC EFFECTS

In all, 204 patients have received Toladryl for periods of time varying from 4 days to 20 months and in amounts ranging from unit doses of 50 mg. to daily doses of 300 mg. (Table VII, A, B, and C). Two of the instances of generalized acute urticaria were followed by the administration of penicillin. One was due to the preoperative use of morphine sulfate in a sensitive individual, one was associated with the use of aureomycin, and the etiology of the others is not clear. It is reasonable to assume that the penicillin and aureomycin reactions would have disappeared with a fair degree of promptness without the drug, but relief was so noticeable after one or two doses that it seems unfair not to credit the medication with part of the relief. The patient who reacted badly to the administration of morphine had previously had two severe reactions to the drug, in each instance associated with high fever, generalized pruritis, facial edema, and large urticarial wheals, the entire process lasting from 10 days to 2 weeks. In the present instance, the urticaria began within 6 hours of the administration of the opiate. The administration of Toladryl was begun approximately 2 hours later. Itching was completely controlled within 24 hours, edema was never a striking feature, the fever did not exceed that to be expected following a pelvic operation, and all vestiges of the urticaria were gone within 3 days. Therefore, despite the fact that such acute urticarial reactions to drugs are almost invariably self-limiting, there is good evidence to show that, in the above cases, the duration of and intensity of such reactions were favorably influenced by the antihistaminic. The etiology of the other acute urticarias was not determined with absolute certainty, but their prompt response to Toladryl leaves little doubt as to its effectiveness.

Angioneurotic edema seemed not to respond as completely or satisfactorily as urticaria to the use of Toladryl. In two instances the cause of the edema was never discovered; these were partially relieved by Toladryl. In the third case, it was believed that an abscessed tooth may have played a part in the production of the allergic reaction. This was discovered after 3 episodes of angioneurotic edema, each of which was fully and rapidly controlled by a unit dose of 50 mg. of Toladryl, but recurred in the first instance 6 hours after such treatment; during the second and third attacks, therefore, the drug was given, 50 mg. every 4 hours for 2 days. Since the removal of the abscessed tooth there have been no recurrences of the condition.

The cause of the *chronic urticarias* was not easily discovered, although gastrointestinal disturbances existed in 2, respiratory allergies were associated in one, and for the remaining no recognizable allergic background existed in the history nor was any discoverable upon physical and laboratory examination. Asthma and urticaria cleared completely in the one subject in whom both were present. In the other subject who obtained complete relief and in one of those who derived partial relief from the drug, there was a prompt recurrence of symptoms whenever it was stopped. Therefore, both of these subjects have continued to use the drug for about 8 months, one remaining completely symptom-free while taking 200 mg. daily, and the other obtaining considerable but not complete relief while using 300 mg. daily. The patient who developed a toxic reaction to the drug was taking 300 mg. at the time. Although the symptoms of his allergy were slightly relieved, drowsiness and dizziness were so distressing that the drug was stopped. He is, therefore, listed as not relieved at all by its administration.

Two of the subjects with *generalized pruritus,* suffering from obstructive jaundice, were only partially relieved by the oral administration of Toladryl in daily doses of 200 mg. The two remaining patients in this group were older people with dry, scaly skin and a condition not uncommonly spoken of as "senile pruritis." In both of these, the condition could be controlled by Toladryl, in one instance with 125 mg., and in the other with 200 mg. daily.

One case of *contact dermatitis* occurred in a laboratory worker who was sensitive to formaldehyde; Toladryl afforded considerable relief despite continued contact. Improvement occurred in a second subject whose condition was due to the employment of an adhesive mixture for the fixation of artificial eyelashes. However, it was never possible to protect this person completely in view of the continued and persistent use of the material. An orderly in the hospital obtained no relief from the irritant effects of soaps and soap powders, contact with which it was impossible for him to avoid. The fourth subject with contact dermatitis was sensitive to the orris root in face powders. Unfortunately for our study, at the same time that she was given Toladryl, a "nonallergic" powder was prescribed. While her symptoms disappeared, the role of the antihistaminics in her therapy was not clear; we have therefore designated this as a negative result.

TABLE VII. RESULTS OF TREATMENT WITH TOLADRYL IN 205 PATIENTS

DIAGNOSIS	NO. PTS.	DAILY DOSE (MG.)		DURATION OF TREATMENT (WKS.)		RESULTS†				TOXIC REACTIONS	
		RANGE	AVG.*	RANGE	AVG.	C.R.	P.R.	N.R.	N.R. % OF TOTAL	NO.	% OF TOTAL
A. Skin Conditions											
Acute urticaria	10	100-200	125	0.5-1.3	0.7	10	0	0	0.0	0	0.0
Angioneurotic edema	3	100-200	150	0.4-1.0	0.6	1	2	0	0.0	0	0.0
Chronic urticaria	5	100-300	200	1.0-30.0	9.2	2	2	1	20.0	1	20.0
Generalized pruritus	4	125-200	200	6.0-32	19.0	2	2	0	0.0	0	0.0
Contact dermatitis	4	100-200	200	2-16	8.3	0	2	2	66.7	1	50.0
B. Respiratory Allergies											
Seasonal allergic rhinitis	88	50-200	100	4-30	8.9	21	57	10	11.4	12	13.6
Nonseasonal allergic rhinitis	45	75-200	100	4-80	16.5	14	22	9	20.0	8	17.8
Bronchial asthma	34	100-300	150	4-58	9.4	7	17	10	29.4	6	17.6
C. Miscellaneous Conditions											
Migraine	4	25-50	25	Unit doses		1	1	2	50.0	1	25.0
Spastic colon	3	50-150	100	1-6	4.0	0	1	2	66.7	1	33.3
Functional dysmenorrhea	1	100-200	--	3‡	3.0	0	0	1	100.0	0	0.0
Hypertension	4	200	200	4-24	20.0	0	2	2	50.0	1	25.0
Grand Total	205					58 (28.4%)	108 (52.6%)	39 (19%)		31 (15.1%)	
Subtotals											
A	167					15	8	3	11.5	2	8.0
B	26					42	96	29	17.3	26	15.5
C	12					1	4	7	58.3	3	25.0

*This represents the dose most commonly used and most frequently satisfactory rather than an actual average of all the dosages used.

†C.R.—Complete relief, i.e., disappearance of all allergic phenomena.

P.R.—Partial relief, usually referring to a disappearance of 50% or more of the distress previously present.

N.R.—No relief—referring to all equivocal results and those in which the drug was a frank failure.

‡Actually, the drug was used for one week premenstrually in each of three succeeding months—100 mg. daily in the first and 200 mg. daily in each of the other trials.

Of the respiratory allergies (Table VII), none has responded so well as *seasonal allergic rhinitis.* Most of our cases of this condition were due to ragweed pollen, others to the pollen of grasses, while one subject, whose condition usually recurred in April, was found to be sensitive to the pollen of the elm tree.

Most of the cases of *nonseasonal allergic* rhinitis were believed to be bacterial in origin and represented instances of sensitivity to bacteria commonly present in the nose and throat. Thirty-six, or 80 per cent, of these subjects obtained complete or partial relief with dosages which rarely needed to exceed 100 mg. per day.

Our efforts to control *bronchial asthma* with antihistaminics appear to have met with more success than those of the majority of investigators. The present data for Toladryl (Table VII B) seem to be in line with this statement. Furthermore, 18 of our 34 subjects had well-advanced secondary pulmonary changes attributable to their asthmatic condition. These have been by all odds the most difficult to control, for of these 18, 9 failed to obtain any relief, whereas in the "uncomplicated" cases only one of 16 was a total failure. In this connection we again emphasize the point that antihistaminics as a class are agents which act in a quantitative manner *to prevent* histamine from attacking sensitized cells and bodily systems. They are totally incapable of correcting reactions once initiated, except for the mild atropine-like action which the diphenhydramine hydrochloride group of derivatives has been shown to exert. Therefore, our results in this field are not recorded in terms of their usefulness when administered to patients who are in the midst of an asthmatic attack, but represent the ability of the selected drug, *when used continually,* to diminish or stop completely the recurrence of attacks. We have now employed diphenhydramine hydrochloride in this manner for several patients over a period of 4 years or more at levels of dosage never lower than 150 mg. daily, and as high as 450 mg. daily without any prolonged evidences of untoward effects and with complete control of the asthmatic state. Toladryl has been used in similar circumstances for 58 weeks. The daily dosage has never exceeded 300 mg., and this amount is usually necessary for rather short periods of time, rarely exceeding one month, while average doses have been in the neighborhood of 150 to 200 mg. daily. While we are fully aware of the fact that this is not curative therapy, we believe it has saved many days of illness and may eventually be shown to play a considerable role in the prevention of the irreversible pulmonary changes that finally lead to serious difficulty in our ability to accomplish anything lasting for the asthmatic individual.

Toladryl has been tried in *migraine, spastic colitis, functional dysmenorrhea,* and *hypertension.* The cases are far too few in number to afford any critical evaluation. However, we believe further trial is warranted in each of the conditions noted, and in other states where releases of histamine may conceivably be concerned in the development or evolution of the medical problem to be treated.

III. TOXIC OR UNTOWARD REACTIONS

In the order of their frequency, the side reactions (Table VIII) observed following the administration of Toladryl were drowsiness, dryness of the mouth

or throat, anorexia, dizziness, tiredness, "jumpiness" in the abdomen, vomiting, disorientation, blurring of vision, and abdominal cramps. In all, 35 of 222 subjects developed some unpleasant symptom. The highest percentage of reactions was observed in those who received 150 mg. of the drug daily. These reactions were so mild that in only 3 patients was it necessary to stop the drug on account of them. In each of these 3 the major symptom was drowsiness; it was noted in 2 when 100 mg. were employed daily and in one when such dosage was 150 mg. If a symptom appeared at any given level of dosage, it was also observed whenever the amount of drug was increased.

TABLE VIII. UNTOWARD REACTIONS IN 222 SUBJECTS RECEIVING TOLADRYL*

CONDITION	NUMBER AND PER CENT OF REACTIONS OBSERVED IN DAILY DOSE OF (MG.)†							
	25 TO 50	75	100	150	200	300	450 OR MORE	TOTAL REACTIONS
NO. OF PATIENTS ON EACH DOSE‡	5	21	157	39	84	15	3	
Drowsiness	0	0	12 (8)	4 (10)	6 (7)	2 (13)	2 (67)	26
Blurring of vision	0	0	0	0	0	0	1 (33)	1
Dizziness	0	0	3 (2)	0	0	0	1 (33)	4
Disorientation	0	0	0	0	0	0	2 (67)	2
Dryness of mouth or throat	0	0	2 (1)	2 (5)	4 (5)	2 (13)	2 (67)	12
Anorexia	0	0	1 (0.5)	1 (2)	2 (2)	1 (7)	1 (33)	6
Vomiting	0	0	2 (1)	0	0	0	0	2
Abdominal cramps	0	0	1 (0.5)	0	0	0	0	1
Jumpiness in the abdomen	0	0	1 (0.5)	1 (2)	0	1 (7)	0	3
Tiredness	0	0	1 (0.5)	1 (2)	1 (1)	1 (7)	0	4
Totals	0	0	23	9	13	7	8	61

*Thirty-five of the 222, or 15.8 per cent, showed some one or more unpleasant symptoms following the administration of Methadryl.

†When "reactions" occurred in any given individual at more than one level of dosage, they are recorded separately for each; hence, the discrepancy between the total number of patients in whom untoward symptoms were observed and the total number of such symptoms.

‡Figures in parentheses represent the approximate percentage of subjects affected at the given level of dosage.

It will be noted that these toxic manifestations are similar in kind to those occurring in connection with the use of diphenhydramine hydrochloride, but are decidedly less frequently encountered and less severe in degree. Their similar nature leads to the conclusion that, in the main, they represent a weak hyoscine-like action.

DISCUSSION

A. *The antihistamine activity.*—The antihistamine activity of Toladryl, a methylated derivative of diphenhydramine hydrochloride has fixed it clearly in the same group of substances as the parent material. The systemic nature of this action was readily demonstrated in human beings by our ability to suppress the dermic wheal and flare reaction to histamine when a sufficient amount of the drug was taken by mouth. This effect afforded no quantitative measurement of its therapeutic usefulness, for while the drug was about 1.5 to 2 times as potent, weight for weight, as diphenhydramine hydrochloride in the control of allergic diseases, it was only about one-half as active in its ability to suppress these dermic reactions to histamine. These facts confirm the oft repeated warning of the allergist that allergic phenomena arise not solely as the result of histamine

releases, important as they may be. Furthermore, it is important to emphasize the point that behavior of a drug in the guinea pig may be considerably at variance with its reaction in the human being. For instance, protection of guinea pigs against histamine mist was afforded by Toladryl in one-half to one-fourth the amounts of diphenhydramine hydrochloride necessary to produce an equivalent protection.[24]

B. *The atropine-like activity.*—The atropine-like activity of Toladryl was certainly less than that of diphenhydramine hydrochloride in both man and lower animals. In the latter, Sharp and his co-workers[24] found Toladryl to be about one-tenth as active as the former. Probably not so great a difference exists in man as determined by topical application to the eye.[1] Unfortunately, the irritant qualities of aqueous solutions of the methylated compound are so great that we did not deem it advisable to use concentrations beyond 0.5 per cent within the conjunctival sac.

C. *Toxicity.*—In human beings, we believe that the true toxic effects of antihistaminic drugs which occur at highest levels of dosage must be distinguished from the "hypersensitive" responses which may be seen in the susceptible individual even after the ingestion of the smallest unit amounts. With Toladryl, the incidence of the latter in a therapeutic range of dosage is about one-fourth to one-fifth that found for diphenhydramine hydrochloride. On the other hand, true toxic reactions, which in some instances represent an intensification of the hypersensitive reaction and its occurrence in a majority of subjects, were seen with Methadryl when 450 mg. were given daily, while they usually were not severe in association with the administration of diphenhydramine hydrochloride until 600 mg. were used daily. In calling attention to these facts, however, it must be pointed out that it is rarely necessary to employ Methadryl therapeutically in a daily dose exceeding 300 mg., even in the most acute urticarias, whereas under similar circumstances we have found it necessary to use as much as 600 mg. of diphenhydramine hydrochloride.

D. *Therapeutic and toxicity indices.*—From the present study, it is evident that Toladryl is from one and one-half to two times as effective weight for weight, as its parent compound, diphenhydramine hydrochloride. This statement seems to follow right down the line of allergic diseases for which the two preparations may be used. For instance, in seasonal hay fever, for which the average subject requires 150 mg. daily of diphenhydramine hydrochloride, 75 to 100 mg. of Toladryl will suffice.

We realize the difficulty encountered in making comparisons between various antihistaminic drugs, both from the standpoint of therapeutic activity and toxicity. The efforts of Loveless and Dworin,[11, 12] Waldbott and Young,[26] Loew,[10] Friedlaender,[4, 5] Feinberg and Bernstein,[3] Gay et al.,[6] McGavack, Weissberg, and Boyd[21] fully illustrate this point.

Unfortunately, even in the comparisons some of these various investigators have reported from their own clinics they have neglected to describe the conditions of the tests, the dosages of the various preparations, the intervals of dosage, the role of placebos, and so forth. We have now made critical comparisons of

the toxicity and therapeutic activity of diphenhydramine hydrochloride, trepelenamine hydrochloride, Neoantergen, Trimeton, and several derivatives of the first mentioned, including Toladryl. In connection with this study it is our opinion that Toladryl is about one-fifth as toxic and from one and one-half to two times as potent therapeutically as its precursor, diphenhydramine hydrochloride.

SUMMARY

1. One hundred thirty-one normal subjects and some of 205 patients have been subjected to extensive clinical study while receiving β-(p-methyl-benzhydryloxy)-ethyldimethylamine hydrochloride (Toladryl). Among the positive effects observed were:

(A) Suppression of the dermal response to histamine in all of 30 subjects studied.

(B) A mild atropine-like action upon the eye in 40 of 60 subjects following the topical application of the drug in a 0.5 per cent aqueous solution.

(C) An improvement in glucose tolerance following the prolonged uses of large doses of Toladryl (450 mg. daily for from one to 4 weeks) in 8 of 11 patients.

(D) A significant lowering of the basal metabolic rate in 19 of 26 subjects who ingested 300 and 450 mg., respectively, for periods of not less than one week each.

2. In daily doses varying from 75 to 600 mg. ingested for periods of time varying from one to 80 weeks no appreciable changes were produced by Toladryl in the following: weight, water balance, pulse rate, blood pressure, electrocardiogram, a variety of fasting blood chemical determinations, the blood count, sedimentation rate of erythrocytes, and urinalysis.

3. Two hundred five patients have received Toladryl over periods of time varying from one day to 80 weeks. Either partial or complete relief was afforded all cases of acute urticaria, angioneurotic edema, and generalized pruritus. More than 85 per cent of the cases of seasonal hay fever were relieved. About 80 per cent of the cases with chronic urticaria and nonseasonal allergic rhinitis were improved, while 70 per cent of the asthmatics were helped in major or minor degree. Among the miscellaneous conditions, in which results of treatment were still less satisfactory, must be included migraine, hypertension, contact dermatitis, spastic colitis, and functional dysmenorrhea.

4. Therapeutically effective amounts of Toladryl varied from unit doses of 25 mg. (e.g., in migraine) to daily doses of 300 mg. (e.g., in acute urticaria). Maintenance doses of the drug have varied from 50 to 200 mg. daily.

5. The histamine-blocking effect of Toladryl may become manifest within 20 to 30 minutes after the first dose is given, but clinical improvement may lag considerably, as in asthma, for, like all other compounds of its class, Toladryl is not capable of reversing or altering the influence of the histamine which has already attached itself to the responding cells or system.

6. In 222 subjects, the over-all incidence of side effects or untoward reactions was 15.8 per cent. No such reactions were observed with daily doses be-

low 100 mg. Qualitatively, these "toxic" symptoms resembled those caused by diphenhydramine hydrochloride, but occurred much less often and were rarely as pronounced. In the descending order of frequency, the most commonly noted were drowsiness, dryness of the mouth and throat, dizziness, tiredness, and vague abdominal complaints. In only 4 of our subjects were these manifestations sufficiently severe to necessitate either a decrease in dose or a cessation of administration. In 2 of these, these side effects became intolerable only when 450 and 600 mg., respectively, were employed daily for a week or more.

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Editorial

DR. HARRY L. ALEXANDER RETIRES AS EDITOR

This issue marks the retirement of Dr. Harry L. Alexander, Clinical Professor of Medicine at Washington University, St. Louis, as Editor of THE JOURNAL OF ALLERGY, a position he has held since the establishment of this publication in 1929. The JOURNAL has been fortunate in having as editor during its formative years a man of such wide experience and reputation in immunology, pathology, physiology, and clinical medicine, who still found time for the exacting details of editorship. His broad knowledge of both the diagnosis and treatment of allergic diseases and the fundamental sciences on which such practice is based, together with fine critical judgment in distinguishing sound new developments from evanescent speculations, have been largely responsible for the progress of the publication.

HARRY L. ALEXANDER

His success is measured by the growth of the JOURNAL from a small beginning to an outstanding position in its field. Although primarily an American enterprise, its circulation has become world-wide, with one-fifth of its paid subscribers in forty-seven other nations on all the continents.

The appreciation of the American Academy of Allergy for this brilliant and faithful work was expressed by a Commemorative Scroll presented at the Sixth Annual Meeting by Dr. Will C. Spain with these words:

> In 1929 Dr. Harry Alexander became the Editor of the JOURNAL OF ALLERGY, its first Editor. For over twenty years he has guided its destinies and is responsible, more than anyone else, for its development into the outstanding national representative publication for allergy that it now is. He has given to it generously of his time, his experience, and his reputation; and we are all proud of the results which he has achieved. Now we have come to the time when Dr. Alexander wishes to retire as active Editor of the Journal. This he plans to do, but we hope sincerely that for many years to come he will give of his experience and judgment to the man who is to take his place.
>
> To you, Harry Alexander, in behalf of the membership of the American Academy of Allergy, as a token of its esteem and gratitude for your many years of faithful and earnest service, I present this Scroll.

The new Editor, with the able advice and aid of the Editorial Board, will attempt to carry on the work of Dr. Alexander by presenting the changing picture of allergy, both as a branch of the practice of medicine and as a biologic concept influencing the pathogenesis of many diseases.

WILLIAM B. SHERMAN